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Omega-3 Fatty Acids and Cardiovascular Disease: An Updated Systematic Review

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None of the investigators has any affiliations or financial involvement that conflicts with the material presented in this report.

This report may periodically be assessed for the urgency to update. If an assessment is done, the resulting surveillance report describing the methodology and findings will be found on the Effective Health Care Program website at: www.effectivehealthcare.ahrq.gov. Search on the title of the report.

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-based Practice Centers (EPCs), sponsors the development of systematic reviews to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. These reviews provide comprehensive, science-based information on common, costly medical conditions, and new health care technologies and strategies.

Systematic reviews are the building blocks underlying evidence-based practice; they focus attention on the strength and limits of evidence from research studies about the effectiveness and safety of a clinical intervention. In the context of developing recommendations for practice, systematic reviews can help clarify whether assertions about the value of the intervention are based on strong evidence from clinical studies. For more information about AHRQ EPC systematic reviews, see www.effectivehealthcare.ahrq.gov/reference/purpose.cfm
AHRQ expects that these systematic reviews will be helpful to health plans, providers, purchasers, government programs, and the health care system as a whole. Transparency and stakeholder input are essential to the Effective Health Care Program. Please visit the Web site (www.effectivehealthcare.ahrq.gov) to see draft research questions and reports or to join an e-mail list to learn about new program products and opportunities for input.

We welcome comments on this systematic review. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by email to epc@ahrq.hhs.gov.

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Abstract

Background: The effect and association of omega-3 fatty acids (n-3 FA) intake and biomarker levels with cardiovascular clinical and intermediate outcomes has remained controversial. This review updates a prior Comparative Effectiveness Review of n-3 FA and clinical and intermediate CVD outcomes.

Objectives: Evaluate the effect and relative effect of n-3 FA on clinical and selected intermediate cardiovascular outcomes and the association between n-3 FA intake and biomarkers and cardiovascular outcomes.

Data sources: MEDLINE, Embase, the Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and CAB abstracts from 2000 (for newly added outcomes and biomarkers) or 2002 (for previously reviewed outcomes) to [19 November 2014, to be updated], and eligible studies from the original reports and relevant existing systematic reviews.

Review methods: We included randomized controlled trials (RCTs) of any n-3 FA (or combination) intake compared to placebo (or lower intake amount) or any other n-3 FA with an outcome of interest conducted in healthy adults or those at risk for cardiovascular disease (CVD) or with CVD. Trials had to report n-3 FA dose. We also included prospective observational studies that analyzed the association between baseline n-3 FA intake or biomarker level and followup cardiovascular outcomes. We required at least 1 year followup for clinical outcomes and at least 4 weeks followup for intermediate outcomes (blood pressure [BP] and plasma lipids). Studies were categorized based on n-3 FA type: total n-3 FA (combined short-chain n-3 FA [ALA] and marine oils [long-chain n-3 FA: EPA, DHA, and DPA]), marine oils, ALA, and SDA.

Results: From 9879 citations (from electronic literature searches and existing systematic reviews), 758 abstracts met basic eligibility criteria; 55 RCTs and 33 longitudinal observational studies (in 144 articles) were included. Most RCTs and observational studies had few risk of bias concerns.

Total n-3 FA: There is low strength of evidence (SoE), based on observational studies, of no association between total n-3 FA intake and stroke death or total incident myocardial infarction. There is insufficient evidence for other outcomes, including from RCTs.

Marine oils, total: There is moderate to high SoE, based on RCTs and observational studies, of beneficial effects of increased marine oil intake to lower triglycerides, raise high density lipoprotein cholesterol (HDL-c), and lower risk of MACE and CVD death, but of no effect on BP, low density lipoprotein cholesterol (LDL-c), all-cause death, and various CVD outcomes. Observational studies provide low SoE of associations between higher marine oil intake and decreased risk of coronary heart disease (CHD) and congestive heart failure (CHF). There is insufficient evidence for other outcomes.

Marine oils, individually: There is low SoE, from observational studies, of no associations between EPA or DHA intake—separately, not in combination—and CHD. There is low SoE, from observational studies, of no association EPA biomarkers and atrial fibrillation, but moderate SoE, from RCTs, of no effect of purified DHA supplementation on BP or LDL-c. There is insufficient evidence regarding effects or associations of DPA or for other outcomes separate from EPA or DHA.

ALA: There is moderate SoE, from RCTs, of no effect of ALA intake on BP, LDL-c, HDL-c, or triglycerides. There is low SoE, from observational studies, of no association between ALA

intake or biomarker level and CHD, CHD death, atrial fibrillation, CHF, total or ischemic stroke. There is insufficient evidence for other outcomes.
Other n-3 FA analyses: There is insufficient direct evidence of comparisons between marine and ALA. There was insufficient evidence for the effects or associations of other n-3 FA intake or biomarkers and CVD outcomes. There is insufficient evidence regarding SDA. There is insufficient evidence regarding comparisons of effect in different populations (including primary versus secondary prevention), among various subgroups of people, between differing sources of n-3 FA, or with different cointerventions.
Conclusions: Most of 55 RCTs evaluated the effects of marine oil supplements compared with placebo on CVD outcomes in populations at risk for CVD or with CVD, while most of 33 observational studies examined the associations between various individual n-3 FA and in combination with each other in relationship to long-term CVD events in generally healthy populations. Compared to the prior report on n-3 FA and CVD, there is more robust RCT evidence on ALA and on clinical cardiovascular outcomes; also, by design there is newly added data on associations between n-3 FA biomarkers and cardiovascular outcomes. However, conclusions regarding the effect of n-3 FA intake on cardiovascular outcomes or associations with outcomes remain unchanged. Marine oils statistically significantly raise HDL-c by a clinically nonsignificant amount and lower Tg in a dose-dependent manner. Marine oils have no significant effect on BP or LDL-c. ALA has no significant effect on intermediate outcomes. Sparse data are available from RCTs on the effect of n-3 FA on clinical CVD outcomes. Observational studies suggest that higher marine oil intake is associated with lower risk of CHD and CHF. No clear differences in effects or associations were evident based on population, demographic features, or cointerventions, although the evidence is limited regarding these comparisons. While the studies generally have few risk of bias concerns, there are important gaps in analyses of interest. Future RCTs would be needed to establish adequate evidence of the effect of n-3 FA on CVD outcomes or to clarify differential effects in different groups of people. Additional participant-level meta-analyses of pooled observational studies are needed to better understand associations of n-3 FA status and clinical outcomes and to attempt to determine n-3 FA dose intake thresholds.

Abbreviations

ACS	acute coronary syndrome
AFib	atrial fibrillation
AHRQ	Agency for Healthcare Research and Quality
AIC	Akaike information criterion (estimation of fit of regression with spline)
ALA	alphalinolenic acid
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CHF	congestive heart failure
CHD	coronary heart disease
CI	confidence interval
CKD	chronic kidney disease
CMS	cardiometabolic syndrome

CVA	cerebrovascular accident (stroke)
CVD	cardiovascular disease
DBP	diastolic blood pressure
DHA	docosahexaenoic acid
DM	diabetes mellitus
DPA	docosapentaenoic acid
EAR	estimated average requirement
EPA	eicosapentaenoic acid
FA	fatty acid(s)
HDL-c	high density lipoprotein cholesterol
HR	hazard ratio
HTN	hypertension
LDL-c	low density lipoprotein cholesterol
MACE	major adverse cardiovascular events
MAP	mean arterial blood pressure
MI	myocardial infarction
n-3 FA	omega-3 fatty acid(s)
n-6 FA	omega-6 fatty acid(s)
ODS	Office of Dietary Supplements
OR	odds ratio
PCI	percutaneous coronary intervention
PUFA	polyunsaturated fatty acids
RCT	randomized controlled trial
SBP	systolic blood pressure
SCD	sudden cardiac death
SDA	stearidonic acid
TEP	Technical Expert Panel
Tg	triglycerides
TOO	task order officer

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Executive Summary

Introduction

Since the first ecological study published in the late 1970s noted a relatively low cardiovascular mortality in a Greenland Eskimo population with high fish consumption,[1] there have been hundreds of observational studies and clinical trials conducted to evaluate the effect of omega-3 fatty acids (n-3 FA) on cardiovascular disease (CVD) and its risk factors and intermediate markers. The n-3 FA (including alphalinolenic acid [ALA], stearidonic acid [SDA], eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA], and docosahexaenoic acid [DHA]) are a group of essential long-chain and very-long-chain polyunsaturated fatty acids (PUFA) that have wide ranges of physiologic effects and play a key role in inflammation regulation. ALA is found in plants, such as leafy green vegetables and nuts, as well as in vegetable oils, such as canola, soy, and flaxseed. SDA can be formed from ALA via Δ6 desaturase, the rate-limiting enzyme in the pathway. Good sources of EPA and DHA in the diet include fish, other seafood, other marine sources (such as algae or krill), and organ meats. EPA can be converted to DPA and vice versa. The conversion rates from ALA to EPA or DHA are highly variable.

Since the publication of the original Agency for Healthcare and Research Quality (AHRQ) n-3 FA systematic reviews in the mid-2000s[2, 3] the topic of n-3 FA and CVD has remained controversial. This topic has been evaluated by several expert panels considering whether recommendations or reference values for intakes of n-3 FA were warranted, either through naturally occurring sources of n-3 FA (e.g., fish consumption) and/or through the use of dietary supplements and fortified foods.[4-7] In 2002, the Institute of Medicine (IOM) considered the evidence inadequate to establish an estimated average requirement (EAR) for n-3 FA. Three other expert reports evaluated the potential health benefits of fish and seafood consumption.[4, 6, 7] Based primarily on the availability of observational study data, these panels consistently suggested that regular consumption of fish and seafood is associated with lower risk of coronary heart disease and cardiac death. These recommendations were based primarily on assumptions of benefits from EPA and DHA and their content in fish and seafood.

There are ongoing concerns in the scientific community regarding systematic biases and random errors in the determination of intakes of n-3 FA from dietary and supplement sources, using currently available assessment tools. Nutrient biomarkers can provide an objective measure of dietary status. However, the correspondence between intake and biomarker concentration not only reflects recent intake but also subsequent metabolism. Current biomarkers used to estimate n-3 FA intake include ALA, EPA, DHA, and, less frequently, SDA and DPA, measured in adipose tissue, erythrocytes, plasma, or plasma phospholipids.[8, 9] Adipose tissue FA are thought to reflect long-term intake, erythrocyte FA are thought to reflect intake over the previous 120 days, and plasma FA are thought to reflect more recent intake.[8]

Scope of the review

The purpose of the current systematic review is twofold: 1) to update earlier reviews of the state-of-the science on the topic of the effects of n-3 FA on CVD[3] and selected cardiovascular risk factors and intermediate markers of CVD,[2] and 2) to collect additional information that will enhance the usefulness of this report for policy and clinical applications. This review updates the outcomes reported in the previous review and expands the scope to include additional CVD outcomes (peripheral vascular disease, congestive heart failure, and

arrhythmias); it updates BP and plasma lipid outcomes and adds incident hypertension; it adds associations between biomarkers of n-3 FA intake and outcomes.

Key questions

The key questions address issues of efficacy (i.e., causal relationships from trials), as well as associations (i.e., prospective observational cohort study associations of n-3 FA intake and/or biomarkers with long-term outcomes; or biomarker associations reported in RCTs). Compared with the key questions from the 2004 reports, the current key questions expand the scope of the review to include additional cardiovascular outcomes (BP, congestive heart failure, and arrhythmias), focus on the intermediate outcomes plasma lipids and BP, add the intermediate outcome hypertension, and include associations between biomarkers of intake and outcomes.

1. What is the efficacy or association of n-3 FA (EPA, DHA, EPA+DHA, DPA, SDA, ALA, or total n-3 FA) exposures in reducing CVD outcomes (incident CVD events, including all-cause death, CVD death, nonfatal CVD events, new diagnosis of CVD, peripheral vascular disease, congestive heart failure, major arrhythmias, and hypertension diagnosis) and specific CVD risk factors (BP, key plasma lipids)?
    - What is the efficacy or association of n-3 FA in preventing CVD outcomes in people
        - Without known CVD (primary prevention)
        - At high risk for CVD (primary prevention), and
        - With known CVD (secondary prevention)?
    - What is the relative efficacy of different n-3 FA on CVD outcomes and risk factors?
    - Can the CVD outcomes be ordered by strength of intervention effect of n-3 FA?

2. n-3 FA variables and modifiers:
    - How does the efficacy or association of n-3 FA in preventing CVD outcomes and with CVD risk factors differ in subpopulations, including men, premenopausal women, postmenopausal women, and different age or race/ethnicity groups?
    - What are the effects of potential confounders or interacting factors—such as plasma lipids, body mass index, BP, diabetes, kidney disease, other nutrients or supplements, and drugs (e.g., statins, aspirin, diabetes drugs, hormone replacement therapy)?
    - What is the efficacy or association of different ratios of n-3 FA components in dietary supplements or biomarkers on CVD outcomes and risk factors?
    - How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by ratios of different n-3 FA—DHA, EPA, and ALA, or other n-3 FA?
    - How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by source (e.g., fish and seafood, common plant oils (e.g., soybean, canola), fish oil supplements, fungal-algal supplements, flaxseed oil supplements)?
    - How does the ratio of n-6 FA to n-3 FA intakes or biomarker concentrations affect the efficacy or association of n-3 FA on CVD outcomes and risk factors?
    - Is there a threshold or dose-response relationship between n-3 FA exposures and CVD outcomes and risk factors? Does the study type affect these relationships?
    - How does the duration of intervention or exposure influence the effect of n-3 FA on CVD outcomes and risk factors?

- What is the effect of baseline n-3 FA status (intake or biomarkers) on the efficacy of n-3 FA intake or supplementation on CVD outcomes and risk factors?

3. Adverse events:
   - What adverse effects are related to n-3 FA intake or biomarker concentrations (in studies of CVD outcomes and risk factors)?
   - What adverse events are reported specifically among people with CVD or diabetes (in studies of CVD outcomes and risk factors)?

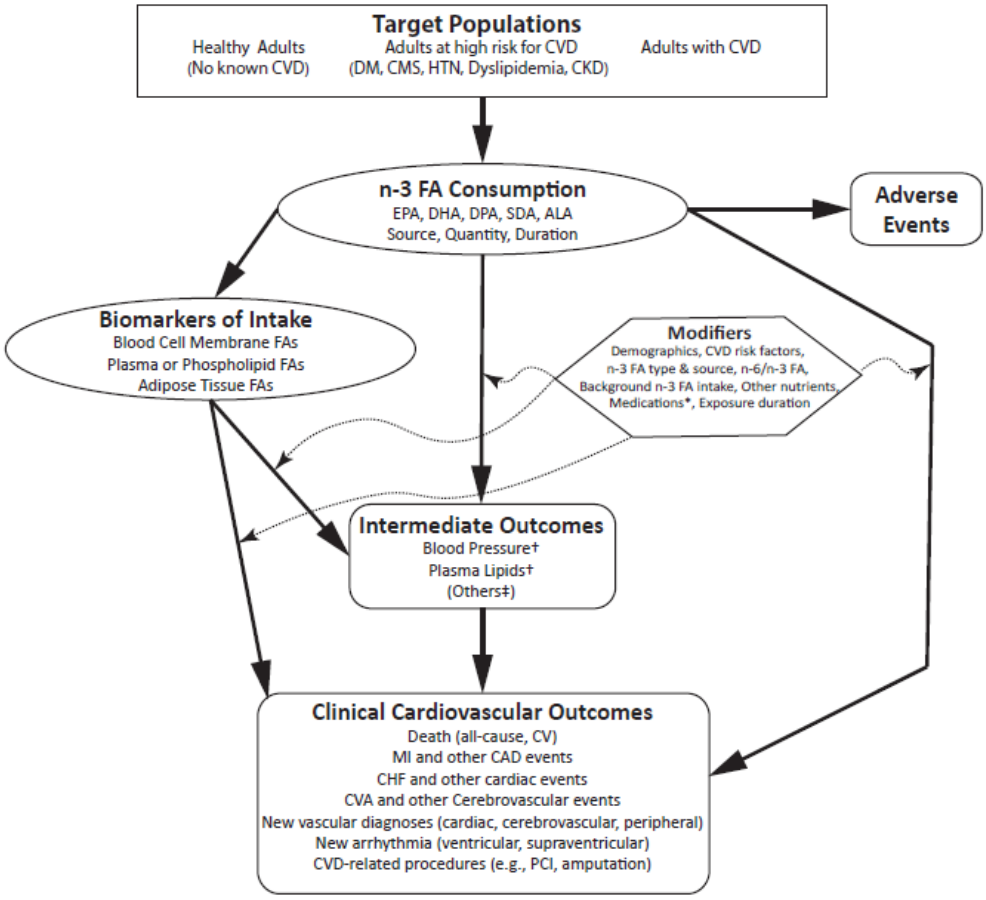
Analytic framework

To guide the assessment of studies that examine the association between n-3 FA intake and cardiovascular outcomes, the analytic framework maps the specific linkages associating the populations of interest, exposures, modifying factors, and outcomes of interest (**Figure A**). The framework graphically presents the key components of well-formulated study questions:

1) Who are the participants (i.e., what is the population and setting of interest, including the diseases or conditions of interest)?
2) What are the interventions?
3) What are the outcomes of interest (intermediate and health outcomes)?
4) What study designs are of value?

Specifically, this analytic framework depicts the chain of logic that evidence must support to link the intervention (exposure to n-3 FA) to improved health outcomes.

Figure A. Analytic framework for omega-3 fatty acid exposure and cardiovascular disease



Legends: This framework concerns the effect of n-3 FA exposure (as a supplement or from food sources) on CVD and cardiovascular risk factors. Populations of interest are noted in the top rectangle, exposure in the oval, outcomes in the rounded rectangles, and effect modifiers in the hexagon.

* Specifically, cardiovascular medications, statins, antihypertensives, diabetes medications, hormone replacement regimens.
† Systolic blood pressure, diastolic blood pressure, mean arterial pressure, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), total/HDL-c ratio, LDL-c/HDL-c ratio, triglycerides.
‡ Many other intermediate outcomes are likely in the causal pathway between n-3 FA intake and cardiovascular outcome, but only blood pressure and plasma lipids are included in the review.

ALA = alphalinolenic acid, CAD = coronary artery disease, CHF = congestive heart failure, CKD = nondialysis-dependent chronic kidney disease, CMS = cardiometabolic syndrome, CVA = cerebrovascular accident (stroke), CVD = cardiovascular disease, DHA = docosahexaenoic acid, DM = diabetes mellitus, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FA = fatty acid, HTN = hypertension, MI = myocardial infarction, n-3 = omega-3, n-6 = omega-6, PCI = percutaneous coronary intervention, SDA = stearidonic acid.

Methods

The present review evaluates the effects of, and the associations between, n-3 FA (EPA, DPA, ALA and n-3 FA biomarkers) and CVD outcomes. The Brown Evidence-based Practice Center (EPC) conducted the review based on a systematic review of the published scientific

literature using established methodologies as outlined in the AHRQ Methods Guide for Effectiveness and Comparative Effectiveness Reviews (Methods Guide).[10]

The review is conducted in parallel with a systematic review of n-3 FA and child and maternal health, conducted by another EPC. Several aspects of the review are being coordinated, including eligibility criteria and search strategies regarding interventions and exposures structure of the reviews, as well as assessments of the studies' risk of bias, strength of the bodies of evidence, and extraction of study characteristics needed to assess causality.

We convened a Technical Expert Panel (TEP) to help refine the research questions and protocol, including the key questions, analytic framework, study eligibility criteria, literature search, and analysis plans.

Literature search

Search strategy

We conducted literature searches of studies in MEDLINE, both the Cochrane Central Trials Registry and Cochrane Database of Systematic Reviews, Embase, and CAB Abstracts from 2002 to [19 November 2014]. We searched publications back to 2000 for the newly added outcomes and for biomarkers of n-3 FA intake. We also included all studies from the original reviews that continued to meet eligibility criteria. Titles and abstracts were independently double-screened to identify articles relevant to each Key Question. We also reviewed reference lists of related systematic reviews. [The search will be updated upon submission of the draft report for peer and public review.]

Inclusion and exclusion criteria

For all key questions, the eligibility criteria are:

Populations

- Healthy adults (≥18 years) without CVD or with low to intermediate risk for CVD
- Adults at high risk for CVD (e.g., with diabetes, cardiometabolic syndrome, hypertension, dyslipidemia, nondialysis chronic kidney disease)
- Adults with clinical CVD (e.g., history of myocardial infarction, angina, stroke, arrhythmia)
- Exclude populations chosen for having a non-CVD or non-diabetes-related disease (e.g., cancer, gastrointestinal disease, rheumatic disease, dialysis)

Interventions/Exposures

- n-3 FA supplements
- n-3 FA supplemented foods (e.g., eggs)
- n-3 FA content in diet (e.g., from food frequency questionnaires)
- Biomarkers of n-3 FA intake
- n-3 FA content of food or supplements must be explicitly quantified. Therefore, studies, such as those of fish diet where only servings per week are defined or Mediterranean diet studies without quantified n-3 FA, are excluded. The n-3 FA quantification can be of total n-3 FA, of a specific n-3 FA (e.g., ALA, purified DHA) or of combined long-chain n-3 FA (EPA, DHA, and DPA, regardless of source; hereafter referred to as marine oil).

- Exclude mixed interventions of n-3 FA and other dietary or supplement differences (e.g., n-3 FA and vitamin E versus placebo; n-3 FA as part of a low-fat diet versus usual diet). However, factorial design (and other) studies that compare (for example) n-3 FA versus control, with or without another intervention (e.g., statins) are included.
- Exclude n-3 FA dose ≥6 g/day
- Exclude weight-loss interventions

Comparators

- Placebo or no n-3 FA intervention
- Different n-3 FA source intervention
- Different n-3 FA concentration intervention
- Different n-3 FA dietary exposure (e.g., comparison of quantiles)
- Different n-3 FA biomarker levels (e.g., comparison of quantiles)

Outcomes

- All-cause death
- Cardiovascular, cerebrovascular, and peripheral vascular events:
    - Fatal vascular events (e.g., due to myocardial infarction, stroke)
    - Total incident vascular events (e.g., myocardial infarction, stroke, transient ischemic attack, unstable angina, major adverse cardiovascular events [MACE]; total events include fatal and nonfatal events; total stroke includes ischemic and hemorrhagic stroke)
    - Coronary heart disease, new diagnosis
    - Congestive heart failure, new diagnosis
    - Cerebrovascular disease, new diagnosis
    - Peripheral vascular disease, new diagnosis
    - Ventricular arrhythmia, new diagnosis, including sudden cardiac death [SCD]
    - Supraventricular arrhythmia, new diagnosis
    - Major vascular interventions/procedures (e.g., revascularization, thrombolysis, lower extremity amputation, defibrillator placement)
- Major CVD risk factors (intermediate outcomes):
    - Blood pressure (new-onset hypertension, systolic, diastolic, and mean arterial pressure)
    - Key plasma lipids (i.e., high density lipoprotein cholesterol [HDL-c], low density lipoprotein cholesterol [LDL-c], total/HDL-c ratio, LDL-c/HDL-c ratio, triglycerides)
- Adverse events (e.g., bleeding, major gastrointestinal disturbance), only from intervention studies of supplements

Timing

- Clinical outcomes, including new-onset hypertension (all study designs): ≥1 year followup (and intervention duration, as applicable)
- Intermediate outcomes (BP and plasma lipids) (all study designs): ≥1 month followup
- Adverse events (all study designs): no minimum followup

Setting

- Community-dwelling (noninstitutionalized) individuals

Study Design

- RCTs (all outcomes)
- Randomized cross-over studies (BP and plasma lipids, adverse events)
- Prospective nonrandomized comparative studies (clinical outcomes, adverse events)
- Prospective cohort (single group) studies, where groups are compared based on n-3 FA intake or intake biomarker values (clinical outcomes)
- Exclude: Retrospective or case control studies or cross-sectional studies (but include prospective nested case control studies). Studies must have measure of intake prior to outcome.

- Minimum sample sizes
    - RCTs
        - We aimed for a minimum of about 25 RCTs for each of the BP and plasma lipid outcomes. We preferentially included RCTs that reported relevant subgroup, interaction, or factorial analyses.
            - For RCTs with BP or lipid outcomes with subgroup, interaction, or factorial analyses, we included parallel design RCTs with a minimum of 30 participants per arm, factorial RCTs with a minimum of 30 participants per n-3 FA intervention, and crossover trials with a minimum of 20 participants.
            - For RCTs with lipid outcomes without subgroup analyses, we included parallel design RCTs with a minimum of 200 participants per arm, factorial RCTs with a minimum of 200 participants per n-3 FA intervention, and crossover trials with a minimum of 100 participants.
            - For RCTs with BP outcomes without subgroup analyses, if followup was ≥6 months, we included all RCTs; if followup was <6 months (≥1 month), we included parallel design RCTs with a minimum of 80 participants per arm, factorial RCTs with a minimum of 80 participants per n-3 FA intervention, and crossover trials with a minimum of 40 participants.
            - For RCTs with CVD event outcomes, we included all RCTs with at least 10 participants per arm.
    - Longitudinal observational studies
        - We aimed for a minimum of about 10 observational studies for each broad clinical outcome (see bullets below) and also for dietary marine oils, dietary ALA, marine oil biomarkers, and ALA biomarkers.
            - For cardiac event outcomes, we included observational studies with at least 10,000 participants.
            - For death outcomes, we included observational studies with at least 10,000 participants.
            - For stroke event outcomes, we included observational studies with at least 3000 participants.
            - For stroke event outcomes, we included observational studies with at least 3000 participants.

        - For arrhythmia event outcomes, we included observational studies with at least 2000 participants.
        - For congestive heart failure event outcomes, we included observational studies with at least 700 participants.
        - For peripheral vascular disease event, incident hypertension, MACE, and revascularization outcomes, we included observational studies with at least 500 participants.
        - We screened smaller sample size observational studies (starting with the largest studies) to include additional studies of ALA biomarkers, regardless of the outcomes analyzed.

    - In all instances, if a study met eligibility criteria for any outcome, we extracted all outcomes of interest from that study; therefore, there are multiple instances of studies being included for an outcome even though the study might not have met study size criteria for that specific outcome.

- English language publications
- Peer reviewed publications

Quality (risk of bias) assessment of individual studies

We assessed the methodological quality of each study based on predefined criteria. For RCTs, we used the Cochrane risk of bias tool[11] and for observational studies we used relevant questions from the Newcastle Ottawa Scale.[12] Additionally, we included nutrition study specific risk of bias questions (e.g., related to uncertainty of dietary assessment measurements).[13-15]

Data synthesis

Statistical analyses were conducted in Stata version 13.1 (StataCorp, College Station, Texas). We conducted random effects model meta-analyses of comparative studies (RCTs) if, for each set of studies with the same outcome and intervention and comparator pair, there were at least six studies. We meta-analyzed observational cohorts when at least four cohorts analyzed the same n-3 FA, measure, and outcome.

Strength and applicability of the body of evidence

We graded the strength of the body of evidence per the AHRQ Methods Guide on assessing the strength of evidence for each outcome.[16] The strength-of-evidence dimensional ratings are summarized in Evidence Profile tables that detail our reasoning behind the overall strength of evidence rating. We qualitatively assessed the applicability within and across studies with reference to whether people in the studies are in the three populations of interest (healthy, at risk, and with CVD), and as pertains to n-3 FA source, type, and dose/exposure.

Peer review and public commentary

A draft version of this report [is being] reviewed by a panel of expert reviewers, including representatives from [pending] and the general public. The reviewers included experts

in [pending]. These experts were either directly invited by the EPC or offered comments through a public review process. Revisions of the draft [will be] made, where appropriate, based on their comments. The draft and final reports [will] also reviewed by the Task Order Officer and an Associate Editor from another EPC. However, the findings and conclusions are those of the authors, who are responsible for the contents of the report.

Results

The literature searches yielded 9676 citations. Reference lists from existing systematic reviews yielded 203 additional citations (which mostly represented articles published before 2002). Of these, 758 abstracts met basic eligibility criteria. As described in the Methods chapter of the full report (under Study Selection), using an evidence map process, we selected 421 articles for full text review, of which 144 articles met eligibility criteria, representing 55 RCTs and 33 longitudinal observational studies.

Across RCTs, the studies generally had few risk of bias concerns. Sixteen of 55 RCTs (29%) had no risk of bias / study quality limitations; an additional 30 RCTs (55%) had one risk of bias limitation. None of the remaining 9 RCTs (16%) had more than four study limitations (of 10 explicitly assessed potential limitations). The most common risk of bias limitation was a lack of intention-to-treat analyses; 14 RCTs (25%) clearly did not conduct intention-to-treat analyses (one of these conducted an intention-to-treat analysis for the outcome death, but not for the lipid outcomes); six additional RCTs (11%) were unclear whether intention-to-treat analyses were conducted. Ten RCTs (18%) did not blind study participants (and three additional RCTs [5%] were unclear whether they blinded participants), often because the intervention was dietary and could not be blinded. However, only four RCTs (7%) clearly did not blind outcome assessors (nine additional RCTs [16%] were unclear regarding outcome assessor blinding). Attrition bias, primarily due to dropout rates greater than 20 percent, was present in 8 RCTs (15%). Other potential biases were less common. A single study had four high risk of bias issues (poor allocation concealment, unblinded participants, unblinded outcome assessors, and likely reporting bias). Three RCTs had three high risk of bias issues each (two studies each with unblinded participants, possible reporting bias, lack of intention-to-treat analyses; one study each with unblinded outcome assessors, attrition bias, and differences in compliance across groups).

Across the observational studies, there were fairly few risk of bias concerns. No study was deemed to have high risk of selection bias (regarding whether the outcome was present at baseline), but for three of 33 studies (9%) it was unclear. Two studies (6%) did not adjust analyses for confounders or other factors. Three studies (9%) did not blind outcome assessors and for another three studies (9%) it was unclear whether they were blinded. Incomplete outcome data analysis was of concern in only one study (3%), but was unclear in another four studies (12%). In three of 26 studies (12%) there was inadequate reporting of the dietary assessment instrument, but only six studies (23%) explicitly estimated n-3 FA from both dietary and supplement sources. The most frequent reporting inadequacy related to whether the ranges and distribution of n-3 FA exposures were fully reported; 15 of 33 studies (45%) did not fully report such data. Only five of 33 studies (15%) had two study limitations (of six explicitly assessed).

The trials of clinical outcomes were almost all conducted in populations at increased risk of CVD, largely related to dyslipidemia, or with CVD. The trials that reported intermediate outcomes (BP and lipoproteins), were conducted in generally healthy, at-risk, and CVD populations. The observational studies, in contrast, were almost all conducted in general

(unrestricted by CVD or risk factors) or healthy populations. Observational studies did not analyze intermediate CVD outcomes.

In this Executive Summary, we present the results by n-3 FA, first summarizing the strength of evidence across studies, then separately summarizing the clinical cardiovascular event outcomes from RCTs, the intermediate cardiovascular outcomes from RCTs, the observational study associations with n-3 FA intake, and the observational study associations with n-3 FA biomarkers. For the interested reader, the main report primarily summarizes the study results first by outcome, then by n-3 FA, then by study design. This summary by n-3 FA is also included in the main report. A listing of effects or associations of n-3 FA and outcomes by the strength of evidence supporting the findings is included at the start of the Discussion section.

Summary by n-3 FA

Total n-3 FA (ALA+EPA+DHA)

Overall, there is insufficient evidence regarding the effect of or association between total n-3 FA (combined ALA and marine oils) and clinical or intermediate outcomes. There is low strength of evidence of no association between total n-3 FA intake and stroke death, and total (fatal and nonfatal) MI (each association based on longitudinal observational studies).

Clinical event outcomes, RCTs

No RCTs reported clinical event outcomes for comparisons of total n-3 FA versus placebo.

Intermediate outcomes, RCTs

Two RCTs that evaluated BP compared combined ALA and marine oil (ALA 1.2 g/d [canola oil] or 2 g ["plant oil"] and 3.6 or 0.4 g EPA+DHA) versus placebo reported on intermediate outcomes. Neither trial found significant effects on BP, LDL-c, HDL-c, Tg, or Total:HDL-c ratio.

Observational studies, intake

Seven studies evaluated total n-3 FA intake. For each outcome there was no consistent (and replicated) significant association between total n-3 FA intake and risk reduction. One of three studies found a significant association between higher total n-3 FA intake and *higher* risk of MACE. In contrast, one of three studies found an association of higher intake with reduced risk of CVD death; one of two studies found a significant association of higher intake with reduced risk of MI death; one study each found significant associations of higher intake with lower risk of death from ischemic stroke or CHF. The other studies found no significant associations. No studies found significant associations with all-cause death (1 study), CHD death (2 studies), total (ischemic and hemorrhagic) stroke death (3 studies), total MI (1 study), total stroke (fatal and nonfatal) (1 study), SCD (1 study), or incident HTN (1 study).

One study found no significant difference in association of total n-3 FA with total CVD death between men and women. Another study found no significant differences in association by different baseline Total:HDL-c ratios between total n-3 FA intake and risk of MI death, total stroke death, or ischemic stroke death.

Observational studies, biomarkers

Three studies evaluated biomarkers for total n-3 FA (combined; plasma, blood, or erythrocyte). One study evaluated numerous outcomes and found significant associations between higher biomarker level and reduced risk of most outcomes (CVD death, CHD death, all-cause death, CHD, ischemic stroke, SCD, AFib, and CHF), but not stroke death, total stroke, or hemorrhagic stroke. In contrast, a second study found no significant association with CHD. The third study found no significant association overall with incident HTN, but did find a significant association in between higher total n-3 FA biomarker levels and lower risk of HTN in younger women (<55 years old) but not in older women.

Marine oil, total: EPA+DHA±DPA

Overall, there is moderate to high strength of evidence of a beneficial effect of increased marine oil intake for selected CVD and intermediate outcomes, but low to high strength of evidence for no effect or association of higher intake and other selected CVD and intermediate outcomes. There is insufficient evidence for most outcomes of interest.

Specifically, there is high strength of evidence, from RCTs, mostly of supplements, that marine oils clinically and statistically significantly lower Tg—possibly with greater effects with higher doses and in people with higher baseline Tg. There is also evidence that they statistically, but arguably not clinically, significantly raise HDL-c. Finally, there is high strength of evidence that marine oil supplementation significantly lowers Total:HDL-c ratio.

There is moderate strength of evidence that marine oil supplementation lowers risk of MACE and CVD death. There is a high strength of evidence of no effect of marine oil on risk of total stroke, but low strength of evidence of no associations of marine oil intake and ischemic or hemorrhagic stroke. There is low strength of evidence for associations between higher EPA+DHA intake and decreased risk of CHD (up to a total intake dose of about 1 g/d) and CHF (up to an intake dose of only 0.2 g/d), based on observational studies. However, there is moderate to high strength of evidence of no effect of (or association between) marine oil intake and all-cause death, MI, AFib, CHF, sudden cardiac death, revascularization, BP, LDL-c, or LDL:HDL-c ratio. There is also low strength of evidence of no effect of marine oil intake and CHD death. There is insufficient evidence for other outcomes.

Clinical event outcomes, RCTs

Regarding clinical event outcomes, 18 trials in populations at increased risk for CVD (2 RCTs) and CVD populations (16 RCTs) mostly found no significant effects of marine oil (EPA+DHA±DPA) versus placebo on specific clinical event outcomes. Across RCTs, EPA+DHA doses ranged from 0.34 to 6 g/d (median 0.866 g/d). Followup ranged from 1 to over 10 years (median 3.9 years).

Two of 15 trials found significantly lower risk of all-cause death with EPA+DHA supplementation (both 0.866 g/d; HR=0.79 and 0.91, vs. placebo), however, the meta-analyzed HR was nonsignificant at 0.97 (95% CI 0.90, 1.05) with no differences across trials by marine oil dose, followup time, or population (CVD, at risk, healthy). Four trials also found no within-study subgroup differences in effect on death for multiple subgroup comparisons.

Eight RCTs each reported on both MACE outcomes and total MI, only one of which found a significant reduction in outcome with 0.866 g/d EPA+DHA versus placebo at 3.9 year followup (HR=0.92, both outcomes). Meta-analysis of MACE (which included a ninth trial of EPA) found a just-significant association (HR=0.95; 95% CI 0.90, 1.00; P=0.047) with no

significant differences across studies by marine oil dose (range 0.4-2 g/d), followup time (range 1-5 y), or population category. Within-study subgroup analyses found a significant effect in women but not men in one trial, but no significant difference in effect between sexes in a second trial and no differences between multiple subgroups in three trials. Meta-analysis of MI (also with the EPA trial) was nonsignificant (HR=0.93; 95% CI 0.83, 1.04), with no significant differences across studies by marine oil dose, followup time, or population category. In one trial, no significant difference in effect was found based on cointervention with B vitamins.

Two of six RCTs found significant effects of 0.866 g/d marine oil (EPA+DHA) versus placebo on risk of CVD death in populations of people with existing CVD. By meta-analysis, there was a near-significant effect (HR=0.91; 95% CI 0.81, 1.01; P=0.073), with no significant differences across studies by marine oil dose, followup time, or population.

Eight RCTs all found no significant effect of EPA+DHA versus placebo with SCD; by meta-analysis (with the EPA trial), summary HR=1.02 (95% CI 0.92, 1.14). Six RCTs also found no significant effect of marine oils with total stroke; by meta-analysis, summary HR=1.02 (95% CI 0.88, 1.19).

All EPA+DHA RCTs that evaluated revascularization (5 trials), CHD death (4 trials), total stroke death (3 trials), AFib (3 trials), and CHF death (1 trial) found no significant effect of marine oils versus placebo. One trial found an effect in participants with diabetes that was not seen in those without diabetes, but no test of interaction was reported. Two trials compared effect of marine oils on AFib in multiple subgroups, finding no significant differences.

Four EPA+DHA RCTs found inconsistent effects on cardiac death, with effect sizes ranging from 0.45 to 1.45. One trial found a statistically significant *reduction* in cardiac death with 0.866 g/d EPA+DHA versus placebo at 3.5 years (RR=0.65; 95% CI 0.51, 0.82); one trial found a statistically significant *increase* in cardiac death with a fish diet with EPA+DHA supplements (0.855 g/d EPA+DHA; HR=1.45; 95% CI 1.05, 1.99), but no significant effect on cardiac death among people only given advice to increase fish intake (by 0.45 g/d EPA+DHA) or in two other trials of 0.96 and 2.6 g/d EPA+DHA. The trial that found increased risk with combined fish diet and EPA+DHA supplementation found no significant difference in effect between multiple sets of subgroups based on drug cointervention.

One of three EPA+DHA RCTs each, found significant effects of reduced angina and CHF incidence with marine oil versus placebo. For angina, across studies EPA+DHA doses ranged from 1.8 to 6 g/d and effect sizes ranged from 0.64 to 1.18; the one trial with a significant effect used a dose of 1.8 g/d. For CHF, across studies doses ranged from 0.866 to 6 g/d and effect sizes ranged from 0.67 to 0.86 (one trial had only one participant who developed CHF); the one trial that found a significant reduction in CHF incidence used a dose of ≥0.85 g/d.

Intermediate outcomes, RCTs

Twenty-two RCTs that compared EPA+DHA to placebo evaluated systolic BP, of which 20 also reported on diastolic BP. Six RCTs were in healthy populations, 11 in populations at risk for CVD, and five in populations with CVD. All trials found no significant difference in BP across EPA+DHA doses of 0.30 to 6 g/d and followup durations of 1 month to 6 years. By meta-analysis (together with two trials of EPA or DHA alone), no significant effects on systolic (summary net difference = 0.15 mmHg; 95% CI −0.17, 0.47) or diastolic (summary net difference = −0.06 mmHg; 95% CI −0.32, 0.21) BP were found. Three of the trials also found no effect on MAP. By meta-regression, no differences in effect across studies were found by marine oil dose, followup duration or population. Three trials directly compared different EPA+DHA

doses and found no differences in effect (1.7 vs. 0.8 g/d; 1.8 vs. 0.9 or 0.45 g/d; 3.4 vs. 1.7 g/d). One trial found no difference in effect between people with normal BP or prehypertension.

Thirty-three marine oil RCTs evaluated LDL-c and HDL-c. Marine oil doses ranged from 0.3 to 6 g/d (median 2.4 g/d) and study followup times ranged from 1 month to 6 years (median 3 months). Meta-analysis of the effect of marine oils on LDL-c found no significant effect (summary net change = 0.3 mg/dL; 95% CI −0.7, 1.2). In contrast, intake of marine oils increased HDL-c by a small, yet statistically significant amount (summary net change = 1.2 mg/dL; 95% CI 0.6, 1.8). Across studies, there were no associations between different marine oil doses, followup durations, or populations (generally healthy, at increased risk for CVD, with CVD) and the effects of marine oil supplementation and either LDL-c or HDL-c. Seven studies found no significant differences in effect within study by EPA+DHA dose. For HDL-c, three trials found no significant difference in effect of marine oil on net change HDL-c between people using statins or not; one or two trials, each, found no significant differences between subgroups based on sex or age. One trial found a significantly larger net increase in HDL-c with marine oil supplementation in a subgroup also randomized to an exercise regimen than in a subgroup without exercise; one of two trials found a significantly larger increase in HDL-c in people with impaired glucose tolerance compared to those with normoglycemia. Seven trials found mostly nonsignificant effects of marine oil (0.4-5 g/d for 1 month to 3 years) on Total:HDL-c ratio; the one trial in healthy participants found significant reductions in Total:HDL-c ratio (−0.5 and −0.8, depending on specific marine oil). The single trial of people with severe hypertriglyceridemia (baseline >500 mg/dL), with subsequent atypically high Total:HDL ratio (8.8), found significant reductions in the ratio with EPA+DHA supplementation (−0.8 and −1.8, depending on dose). The other five trials found no significant net changes in Total:HDL ratio (−0.2 or −0.3 in three trials of at risk populations; −0.06 in people with CVD). Trials that compared purified EPA to purified DHA supplementation or that compared different doses of EPA+DHA supplementation found no differences in effects of marine oil supplementation on either LDL-c or HDL-c.

Thirty-four included RCTs mostly found significant effects of supplementation of marine oils (0.3-6 g/d; median 2.4 g/d for 1 month to 6 years; median 3 months) on Tg levels. Meta-analysis found a summary net change of −23 mg/dL (95% CI −29, −18), with no significant difference in effect based on population (generally healthy, at risk, or with CVD) or followup time across studies. By metaregression, each increase in mean baseline Tg concentration by 1 mg/dL was associated with a greater net decrease in Tg concentration of −0.12 mg/dL (95% CI −0.22, −0.03; P=0.013); each increase of EPA+DHA dose by 1 g/d was also associated with a greater net decrease in Tg concentration of −6.8 mg/dL (95% CI −11.4, −2.2; P=0.005). Across studies, there was no EPA+DHA dose, above which the slope of the association changed (i.e., no clear inflection point was found at any dose). Five of six trials found no significant difference in Tg change by EPA+DHA dose, but across trials all doses of 3.4 and 4 g/d lowered Tg concentration by at least 30 mg/dL more than lower doses (1-2 g/d), while all pairwise comparisons of lower doses (1.7-3 g/d) to even lower doses (0.7-2.25 g/d) found much smaller differences between doses (−17 to 6 mg/dL). Two trials both found significantly larger Tg concentration lowering effects of EPA (3.6 or 3.3 g/d) than DHA (3.8 or 3.7 g/d). No significant differences were found based on statin use (4 trials), vitamin C use (1 trial), concurrent high or low linoleic acid diet (1 trial), concurrent general dietary advice (1 trial), or age (1 trial). One trial found a significantly larger effect on Tg among people also taking a multivitamin. One trial found a larger decrease in Tg with higher dose EPA+DHA (1.8 g/d) in men than in women, but no significant difference in decrease in Tg between sexes at 0.8 g/d. One trial found no

significant difference in effect between people with impaired glucose tolerance and those with noninsulin dependent diabetes, but among those with diabetes, a larger effect was found in those with baseline HDL-c ≤35 mg/dL compared to higher levels.

Observational studies, intake

Twenty-one observational studies evaluated associations between total EPA+DHA±DPA intake (including dietary and supplement intake) and numerous clinical outcomes. Only eight (38%) of these found significant associations with any clinical outcome. For CHD, by meta-analysis, overall there is a near significant association between higher marine oil intake and lower risk of CHD across a median dose range of 0.038 to 3.47 g/d; the best-fit curve found a change in slope (between g/d and risk of CHD) at 1.0 g/d. Below this threshold (from about 0.038 to 1.0 g/d), cohorts of people with higher marine oil intake had lower risk of CHD, but above this threshold (1.0 g/d) there was no significant association between marine oil intake dose and risk of CHD. However, using intake thresholds from 0.2 to 1.4 g/d resulted in similar findings (protective associations at lower intake, no significant association at higher intake). For total stroke, by meta-analysis, there was no significant association across a median dosage range of 0.025 to 0.6 g/d. For ischemic stroke, by meta-analysis, there is a just-significant association between higher marine oil intake and *higher* risk of ischemic stroke across a median dosage range of 0.025 to 0.6 g/d. However, allowing for a change in the slope of the association between marine oil intake and risk of ischemic stroke (across studies) yielded a nonsignificant decreasing risk of with higher intake below intake of 0.3 g/d and a nonsignificant increasing risk above this threshold. Similar results were found with thresholds between 0.1 and 0.5 g/d. For hemorrhagic stroke, by meta-analysis, no significant association was found between EPA+DHA±DPA intake and hemorrhagic stroke. For all-cause death, no studies found significant associations between intake and all-cause death (2 studies).

A minority of studies found significant associations of decreased risk of other outcomes with increasing intake of EPA+DHA±DPA: MACE (1/2 studies), all-cause death (1/3 studies), CVD death (1/4 studies), CHD death (3/7 studies), MI (1/2 studies), incident CHF (1/5 studies), and AFib (1/3 studies). No studies found significant associations with cardiac death (1 study), total stroke death (1 study), ischemic stroke death (1 study), coronary revascularization (1 study), SCD (2 studies), and incident HTN (1 study). One study each analyzed MI death and ischemic stroke death and found a significant association between increased intake and lower risk.

Observational studies, biomarkers

Five studies evaluated combined EPA+DHA±DPA biomarkers, including adipose tissue, cholesteryl ester, erythrocyte, phospholipid, and plasma n-3 FA levels. Of the outcomes evaluated, none was analyzed by more than two studies. One study each found no significant association between various biomarker levels and MI, hemorrhagic stroke, total stroke (P=0.07), or cardiac death. One study found a significant association between higher phospholipid EPA+DHA+DPA levels and incident CHD. Another found a significant association between higher adipose EPA+DHA+DPA levels and acute coronary syndrome (ACS) in men, but not in women. Two studies each evaluated CHF, ischemic stroke, and MACE. For each outcome, only one of the studies found significant associations with EPA+DHA (or EPA+DHA+DPA) biomarker levels. In one of the studies of CHF, phospholipid EPA+DHA+DPA level was associated with higher risk of each outcome in women only but cholesteryl ester EPA+DHA+DPA levels were not associated in either sex.

EPA

For the most part, there is insufficient evidence regarding the effect of, or association with, EPA (specifically) and CVD clinical and intermediate outcomes. There is low strength of evidence of no association between EPA intake and CHD and between EPA biomarkers and AFib.

Clinical event outcomes, RCTs

Regarding clinical event outcomes, one trial in an at risk population (with dyslipidemia), found that after 5 years people taking purified EPA 1.8 g/d had significantly lower risk of MACE and angina compared with placebo, but no significant difference in CHD death, coronary revascularization, SCD, or MI. Subgroup analysis for CHD death found no clear difference between the subgroup who also had CVD (20% of the participants) versus the majority in the study without a history of CVD.

Intermediate outcomes, RCTs

Two RCTs evaluated BP or lipid outcomes. One trial of purified EPA 3.8 g/d versus placebo found no significant effect of EPA supplementation on systolic BP, diastolic BP, or MAP. This trial and another of EPA 3.3 g/d found no significant effect of EPA supplementation on LDL-c or HDL-c. Both trials, however, found significant net reductions in Tg concentration (−42 and −23 mg/dL). The trial of EPA 3.8 g/d also found a significant reduction in Total:HDL-c ratio (−0.2).

Observational studies, intake

Eight studies evaluated associations between estimated total EPA intake and clinical outcomes. No outcome was evaluated by more than two studies. One study each found no significant association between EPA intake and ACS, ischemic stroke, or total stroke death. One study found a significant association between higher EPA intake and lower ischemic stroke death in healthy adults (in quantiles with median EPA intake >0.07 g/d in men and >0.06 g/d in women), but no association with hemorrhagic stroke death. One study found a significant association between higher EPA intake and lower risk of all-cause death (>0.01 g/d) in healthy adults; another study found a significant association with lower risk of MACE in healthy adults (>0.09 g/d). Two studies, each, found no significant associations between EPA intake and incident CHD (although P=0.06 in one) or CHD death. For both incident HTN and CVD death, one of two studies found significant associations between higher EPA (0.02 g/d for HTN and 0.01 g/d for CVD death) intake and lower risk of HTN and CVD death; the other studies found no such associations.

Observational studies, biomarkers

Ten studies evaluated associations between various EPA biomarkers and clinical outcomes. Three studies of healthy adults evaluated incident CHD. Two of these studies found that increased plasma or phospholipid EPA levels were associated with reduced risk of CHD; the third study found no significant association between blood EPA levels and CHD risk. Three studies (two in healthy adults, one in people with hypercholesterolemia) evaluated MACE. T; the study of people with hypercholesterolemia found an association of reduced MACE risk with higher plasma EPA, as did one study of phospholipid EPA in healthy adults. The third study found no significant association between erythrocyte EPA and MACE in healthy adults. Three

studies, two in healthy adults and one in adults with a history of MI, evaluated CHF; in one study of healthy adults, higher plasma EPA was associated with reduced CHF risk, but the other study of healthy adults found no association with phospholipid or cholesteryl ester EPA and CHF risk. The study in people with a history of MI also found an association between higher blood EPA level and lower CHF risk. In this latter study, significant interactions were found for sex (no association was seen in women, in contrast with a significant association in men), statin use (those on statins had no association, in contrast with those not on statins), and baseline HDL-c level (those with higher HDL-c, ≥40 mg/dL, had no association, in contrast with those with lower HDL-c, <40 mg/dL). No interactions were found for age, use of angiotensin receptor blocker drugs, use of beta blocker drugs, diabetes, dyslipidemia, baseline LDL-c, hypertension, glomerular filtration function, or hypertriglyceridemia.

One of three studies found a significant association between higher EPA biomarkers (plasma EPA) and lower risk of death in healthy adults, but a second study of plasma EPA in healthy adults found no such association; nor did a study of blood EPA in people with a history of MI. One of two studies of plasma EPA in healthy adults found a significant association of higher plasma EPA with lower risk of CVD death. Two studies found no significant association between EPA biomarkers and ischemic stroke. One study found a significant association between erythrocyte EPA and incident HTN. One study each found no associations between EPA biomarker levels and ACS, AFib, SCD, MI, hemorrhagic stroke, total stroke, cardiac death, CHD death, or total stroke death.

DHA

For the most part, there is insufficient evidence regarding the effect of, or association with, DHA and CVD clinical and intermediate outcomes. There is moderate strength of evidence of no effect of purified DHA supplementation on BP or LDL-c and low strength of evidence of no association between DHA intake and incident CHD (from observational studies).

Clinical event outcomes, RCTs

No trial that reported clinical event outcomes evaluated DHA alone.

Intermediate outcomes, RCTs

Two trials compared purified DHA (3.6 and 2 g/d) to placebo and found no significant effects on systolic or diastolic BP. One of the trials also found no significant effect on MAP. Three trials of DHA (3.7, 3.6, or 2 g/d) also found no significant effect compared to placebo on LDL-c or HDL-c. Two trials (3.7 and 3.6 g/d) reported on Tg concentration changes and both found significant net reductions compared to placebo with DHA supplementation (−27 and −29 mg/dL). The trial of DHA 3.6 g/d also found a significant reduction in Total:HDL-c ratio (−0.3) compared to placebo.

Observational studies, intake

Eight studies evaluated the association between estimated total DHA intake (specifically) and risk of clinical outcomes. No outcome was reported in more than two studies. Two studies found significant associations between higher DHA intake and lower risk of incident HTN in healthy young adults (18-30 years old in one study; 39-54 year old women in a subgroup of one study), but not in an older subgroup (55-89 years old in one study). In the study of young adults, a significant association was found in quartiles with DHA intake >0.06 g/d compared to quartiles

with lower intake. One of two studies of healthy adults found an association of lower CVD death with DHA intake >0.15 g/d. Two studies each found no association with CHD death or incident CHD (in populations with a broad range of ages, from 20-69 to 45-84 years old). One study each found significant associations of higher DHA intake with increased incidence of MACE (>0.15 g/d DHA), ischemic stroke death (>0.15 g/d), and all-cause death (>0.02 g/d). In one study each, no associations were found with ACS, ischemic stroke, hemorrhagic stroke death, or total stroke death.

Observational studies, biomarkers

Eleven studies evaluated various DHA biomarkers and their associations with clinical outcomes. Overall, a high proportion of observational studies found statistically significant associations between higher DHA biomarker levels and decreased risk of outcomes. Four studies evaluated MACE (with various definitions); two found significant associations between higher DHA biomarker levels (phospholipid and adipose DHA) and lower risk of MACE in healthy adults. The other two studies found no association, one in hypercholesterolemic adults on statins (plasma DHA) and one in healthy adults (erythrocyte DHA). Two of three studies in healthy adults found significant associations between higher plasma or phospholipid DHA and lower CHD risk; the third study, also in healthy adults, found no association with blood DHA. Three studies evaluated CHF. One found associations between higher cholesteryl ester and phospholipid DHA and lower risk of incident CHF in healthy women, but not healthy men (whether the associations were significantly different between women and men was not reported). One study found that overall, there was no significant association of CHF with blood DHA in adults with a history of MI, but that there were significant associations in subgroups of people, such that significant association between higher blood DHA and lower risk of CHF were found in in a population with a history of MI not taking a statin (P interaction with statin use = 0.003), ≥65 years old (P interaction = 0.051), with LDL-c ≥100 mg/dL (P interaction = 0.068), and with HDL-c ≤40 mg/dL (P interaction = 0.096). Three studies also evaluated all-cause death, two of which found significantly lower risk of death with higher plasma DHA (healthy adults) and blood DHA (in people with a history of MI who are not taking statins); another study of healthy adults found no association with plasma DHA.

Two studies found near significant associations between higher cholesteryl ester DHA, phospholipid DHA, and plasma DHA and lower risk of ischemic stroke in healthy adults. One study of healthy adults found an association between higher plasma DHA and lower risk of CVD death (both studies evaluated plasma DHA). One study each found significant associations between higher DHA biomarker levels and lower incidence of AFib, SCD, and CHD death (all plasma DHA in healthy adults). One study found a significant association between higher adipose DHA and lower risk of ACS in healthy men, but not healthy women. Another study found a significant association between higher erythrocyte DHA and lower risk of incident HTN in healthy women aged 39 to 54 years, but not in women older than 54 years. One study found no significant associations between plasma DHA and both total stroke and total stroke death in healthy adults. One study, each, found no significant associations with MI, hemorrhagic stroke, or cardiac death.

DPA

Overall, there is insufficient evidence regarding effect of or association between DPA (specifically) and CVD clinical and intermediate outcomes. There is low strength of evidence of an association between higher DPA biomarker levels and lower risk of AFib.

RCTs

No eligible RCTs compared purified DPA formulations versus placebo.

Observational studies, intake

Two observational studies evaluated estimated total DPA intake (specifically). One study found no significant association between DPA intake and ACS in either healthy men or women. The other found significant associations between higher DPA intake and both incident CHD and MACE in healthy adults, in both instances with a significant association in the quartile with DPA intake >0.04 g/d.

Observational studies, biomarkers

Seven studies evaluated the association of various DPA biomarkers with clinical outcomes, all in healthy adults. No outcome was evaluated by more than three studies. One study in adults age ≥65 years evaluated several clinical outcomes. It found significant associations between higher plasma DPA and lower risks of all-cause and CVD death, near-significant associations with incident CHF (P=0.057) and total stroke death (P=0.056), but no significant associations with AFib, SCD, hemorrhagic, ischemic, or total stroke, or CHD death. For both CHD and MACE, one study found a significant association between higher blood DPA and lower incident CHD, but two studies found no association with plasma or phospholipid DPA. Similarly, one study found a significant association between higher adipose tissue DPA and lower MACE risk, but two found no association with phospholipid or erythrocyte DPA. One study evaluated ACS and found a significantly lower risk in men with higher adipose tissue DPA, but no significant association in women. One study evaluated incident HTN and found a significant association of higher erythrocyte DPA and lower HTN risk in younger women (39-54 years old), but not older women (55-89 years old). One study found no significant association with cardiac death.

SDA

Overall, there is insufficient evidence regarding effect of or association between SDA (specifically) and CVD clinical and intermediate outcomes.

RCTs

No eligible RCTs compared purified SDA formulations versus placebo.

Observational studies

A single eligible observational study in healthy men evaluated baseline erythrocyte SDA and clinical outcomes. Erythrocyte SDA was not significantly associated with either MACE or cardiac death.

Marine oil comparisons

There is insufficient evidence regarding comparisons of specific marine oils (e.g., EPA vs. DHA).

Clinical event outcomes, RCTs

No trial that reported clinical event outcomes compared marine oils.

Intermediate outcomes, RCTs

Two trials that compared marine oil (EPA 3.8 g/d vs. DHA 3.6 g/d; EPA+DHA 3.4 and 1.7 g/d vs. EPA 1.8 g/d) found no significant differences in effect on BP, LDL-c, HDL-c, Tg, or Total:HDL-c ratio.

ALA

There is moderate strength of evidence of no significant effect of ALA intake on BP, LDL-c, HDL-c, or Tg. There is low strength of evidence of no association between ALA intake or biomarker level and CHD or CHD death, AFib, CHF, total and ischemic stroke, based on observational studies. There is insufficient evidence regarding other outcomes.

Clinical event outcomes, RCTs

Two RCTs that evaluated ALA supplementation versus placebo reported clinical event outcomes, one in participants with CVD and one in healthy participants. All analyses were nonsignificant for all-cause death (2 trials) and (from one trial each) MACE, CVD death, cardiac death, CHD death, CHF death, total MI, incident angina, total stroke, and SCD. Within-study subgroup analyses revealed no significant differences in effect for various subgroups for MACE (1 trial) or for subgroups with or without diabetes for CHD death (1 trial).

Intermediate outcomes, RCTs

Five ALA RCTs evaluated BP, with doses ranging from 1.4 to 5.9 g/d for 1 to 3.4 years. All found no significant effect on systolic or diastolic BP, mostly with wide confidence intervals. One of the trials found no significant difference in effect of ALA on BP between a subgroup with hypertension and the study population as a whole. Another trial found no significant difference in effect between 1.4 and 5.9 g/d ALA. No trial reported on MAP.

Four of the trials reported no significant effects of ALA on LDL-c, HDL-c, Tg, or Total:HDL-c ratio (2 trials). No differences in effect were found in the one trial that compared 1.4 and 5.9 g/d ALA. No trial reported on LDL:HDL-c ratio.

Observational studies, intake

Thirteen observational studies evaluated ALA intake. One of these was a pooling of 11 prior studies (the pooled studies are not included in duplicate for the outcomes evaluated by the pooling study). The large majority of analyses found no significant associations; only two studies found any significant associations between higher ALA intake and clinical outcomes. Two studies found significant associations between higher ALA intake and reduced all-cause death (>2.2 g/d in healthy adults; also in healthy men but insufficient data were reported regarding a dose threshold). One of two studies found a significant association between higher ALA intake (>0.6 g/d) and SCD in healthy women but not in a subset of women with CVD; the second study

found no significant association in healthy adults. One of two studies found a significant association between higher ALA intake (unclear threshold) and lower risk of CVD death in younger men (35-57 years old), but another study found no association in older men (≥65 years old). Among four analyses, representing 14 total studies, only one study (not the pooled study) found a significant association between higher ALA intake and lower CHD death risk (unclear threshold). For all other analyzed clinical outcomes, no significant associations were found with ALA intake, including incident CHD (6 analyses of 16 studies total), CHF (4 studies), CVD (3 studies), MACE (2 studies), hemorrhagic and ischemic stroke (2 studies each), AFib (1 study), and HTN (1 study).

Observational studies, biomarkers

Eight studies evaluated various ALA biomarkers. Almost all analyses found no significant associations between ALA biomarkers and clinical outcomes. No outcome was evaluated by more than three studies. For CHF, one study found a significant association between higher plasma ALA and CHF in healthy men, but two other studies found no significant associations in healthy adults across levels of plasma, cholesteryl ester, or phospholipid ALA. One of two studies found a significant association between higher plasma ALA and lower risk of CVD death, but the other study found no significant association with plasma ALA in healthy adults. No significant associations were found for ischemic stroke (3 studies), incident CHD, hemorrhagic and total stroke (2 studies each), MACE (2 studies), all-cause death (2 studies), or AFib, SCD, incident HTN, cardiac death, or CHD death (1 study each).

Marine oil versus ALA

There is insufficient evidence of direct comparisons between marine oil and ALA intake on CVD outcomes. Across studies, the indirect comparison between marine oil and ALA is unclear, largely because there are insufficient studies that evaluated ALA. However, for Tg and HDL-c, where there is high strength of evidence of significant effects of higher dose of marine oil improving Tg and HDL-c, there is moderate strength of evidence of no effect of ALA intake on these intermediate outcomes.

Clinical event outcomes, RCTs

No trial that reported clinical event outcomes directly compared marine oils and ALA.

Intermediate outcomes, RCTs

One trial that compared two doses of EPA+DHA (1.7 and 0.8 g/d) with ALA 4.5 g/d found no differences systolic or diastolic BP at 4 months. Across trials, there was no evidence that intake of any type of n-3 FA had an effect on BP; no difference in effect was apparent between marine oil and ALA trials.

Two trials that compared EPA+DHA (0.8 and 1.7 g/d in one trial, 0.4 g/d in the other) to ALA (4.5 g/d [rapeseed oil margarine] and 2 g/d ["plant oil" margarine], respectively) for 6 months and 3.4 years found no differences between intake of n-3 FA and LDL-c, HDL-c, or Tg levels. Neither trial reported on lipid ratios. No evident differences were found across trials between marine oils and ALA for their nonsignificant effects on LDL-c and HDL-c. In contrast with the two trials that directly compared EPA+DHA and ALA, 32 marine oil (versus placebo) trials fairly consistently found significant effect on Tg reduction in contrast with the four ALA (versus placebo) trials, which mostly had imprecise estimates of effects on Tg.

Discussion

Overall summary of key findings

In this systematic review we identified 55 eligible RCTs (in 85 publications) and 33 eligible prospective longitudinal and nested case-control studies (in 59 publications) for inclusion, based on prespecified eligibility criteria. Most of the RCTs evaluated the effects of marine oil supplements (EPA+DHA) compared with placebo on clinical CVD outcomes in populations at risk for CVD or with CVD, while most of the observational studies examined the associations between intake of various individual n-3 FA, alone and in combination with each other, in relation to long-term CVD events in generally healthy populations. The RCTs of intermediate CVD outcomes (BP and lipids) were conducted in all three populations of interest (generally healthy, at risk for CVD—primarily due to dyslipidemia, or with CVD). However, none of the observational studies evaluated BP or lipids.

The main findings of the studies, regarding effect or association of increased n-3 FA intake or biomarker level and outcomes are summarized in the following tables. **Table A** includes analyses of n-3 FA and outcome pairs for which there is evidence supporting an effect or association of increased n-3 FA intake and lower risk of a CVD outcome or an improved cardiovascular risk factor.

Table A. Main findings of high, moderate, or low strength of evidence of significant effects or associations between n-3 FA and outcomes

There is **high** strength of evidence for the following effects or associations of *increased* n-3 FA intake or biomarker levels and *lower* cardiovascular risks or events:

- Marine oil supplementation (or increased intake) and an increase in HDL-c
    - RCTs (of mostly supplements)
    - Summary net change in HDL-c: 1.2 mg/dL (95% CI 0.6, 1.8)
- Marine oil supplementation (or increased intake) and a decrease in triglycerides (Tg)
    - RCTs (of mostly supplements)
    - Summary net change in Tg: -23 mg/dL (95% CI -29, -18)
- Marine oil supplementation (or increased intake) and a decrease in total or LDL-c to HDL-c ratio
    - RCTs (of mostly supplements)
    - Summary net change in LDL:HDL-c ratio: −0.3 (95% CI −0.4, −0.1)

There is **moderate** strength of evidence for the following effects or associations of *increased* n-3 FA intake or biomarker levels and *lower* cardiovascular risks or events:

- Marine oil supplementation (or increased intake) and a lower risk of major adverse cardiovascular events (MACE)
    - RCTs (of mostly supplements); however, observational studies found no association
    - Summary effect size (RCTs): 0.95 (95% CI 0.90, 1.00)
- Marine oil supplementation (or increased intake) and a possibly lower risk of cardiovascular disease (CVD) death
    - RCTs (of mostly supplements); however, observational studies found no association
    - Summary effect size (RCTs): 0.91 (95% CI 0.81, 1.01)

There is **low** strength of evidence for the following effects or associations of *increased* n-3 FA intake or biomarker levels and *lower* cardiovascular risks or events:

- Marine oil increased intake and a lower risk of coronary heart disease (CHD)
    - Observational studies (of total dietary intake), supported by a single study of n-3 FA biomarkers
- Marine oil increased intake (up to about 0.2 g/d) and a lower risk of congestive heart failure (CHF); no association between intake and CHF risk for intakes >0.2 g/d
    - Observational studies (of total dietary intake); however RCTs of supplements found no effect and biomarker associations studies found no association
    - Summary HR (per g/d): 0.45 (95% CI 0.28, 0.72) (observational studies) for intake between about 0 and 0.2 g/d

Table B includes analyses of n-3 FA and outcome pairs for which there is evidence supporting no effect or association of n-3 FA intake (or biomarker level) and outcomes. Analyses of n-3 FA and outcome pairs not included in the boxes provided insufficient evidence.

Table B. Main findings of high, moderate, or low strength of evidence of no significant effects or associations between n-3 FA and outcomes

There is **high** strength of evidence of *no effect or association* of n-3 FA intake or biomarker level and the following outcomes:

- Marine oil (long-chain n-3 FA, mostly EPA and DHA) intake and all-cause death
  - RCTs (of mostly supplements) supported by observational studies (of total dietary intake)
- Marine oil intake and total stroke (fatal and nonfatal ischemic and hemorrhagic stroke)
  - RCTs (of mostly supplements) supported by observational studies (of total dietary intake)
- Marine oil intake and sudden cardiac death (SCD)
  - RCTs (of mostly supplements) supported by an observational study (of total dietary intake)
- Marine oil intake and coronary revascularization
  - RCTs (of mostly supplements) supported by an observational study (of total dietary intake)
- Marine oil intake and systolic or diastolic blood pressure
  - RCTs (of mostly supplements)
- Marine oil intake and LDL-c
  - RCTs (of mostly supplements)

There is **moderate** strength of evidence of *no effect or association* of n-3 FA intake or biomarker level and the following outcomes:

- Marine oil intake and myocardial infarction
  - RCTs (of mostly supplements) supported by an association study (of total dietary intake)
- Marine oil intake and atrial fibrillation
  - RCTs (of mostly supplements); observational studies of intake were inconsistent
- Purified DHA supplementation and systolic or diastolic blood pressure
  - RCTs
- Purified DHA supplementation and LDL-c
  - RCTs
- ALA intake and systolic or diastolic blood pressure
  - RCTs (of mostly supplements)
- ALA intake and lipoproteins (LDL-c, HDL-c) or Tg
  - RCTs (of mostly supplements)

There is **low** strength of evidence of *no effect or association* of n-3 FA intake or biomarker level and the following outcomes:

- Total n-3 FA intake and stroke death
  - Observational studies (of total dietary intake)
- Total n-3 FA intake and myocardial infarction death
  - Observational studies (of total dietary intake)
- Marine oil intake and CHD death
  - RCTs (of mostly supplements); observational studies of intake were inconsistent
- Marine oil intake and ischemic or hemorrhagic strokes
  - Observational studies (of total dietary intake)
- EPA intake and CHD
  - Observational studies (of total dietary intake)
- EPA biomarkers and atrial fibrillation
  - Observational studies
- DHA intake and CHD
  - Observational studies (of total dietary intake)
- DPA biomarkers and atrial fibrillation
  - Observational studies
- ALA intake and CHD or, separately, CHD death
  - Observational studies (of total dietary intake); CHD death finding supported by one RCT (of supplementation)
- ALA intake and atrial fibrillation

- Observational studies (of total dietary intake)
- ALA intake and CHF
  - Observational studies (of total dietary intake), supported by one RCT (of supplementation)

The overall findings for the effects of marine oil supplements on intermediate CVD outcomes remain largely unchanged since the original report. In this update, there were no significant effects found in 22 RCTs that compared marine oils (0.3-6 g/d) on SBP or DBP compared with placebo. Thirty-three RCTs evaluated LDL-c and HDL-c. Meta-analysis of the effect of marine oils on LDL-c found no significant effect. In contrast, marine oils increased HDL-c by a small, but statistically significant amount (summary net change = 1.2 mg/dL; 95% CI 0.6, 1.8). The clinical significance of this small increase in HDL-c on CVD outcomes is unclear. For both lipid outcomes, no differences in effect across studies were found by marine oil dose, followup duration or population. The strongest effect of marine oils (0.3-6 g/d) was found among the 34 RCTs of Tg. Meta-analysis found a summary net change of −23 mg/dL (95% CI −29, −18), with no significant difference in effect based on population or followup time across studies. However, across trials, the effect was dose-dependent and also dependent on the studies' mean baseline Tg values. By metaregression, each increase of EPA+DHA dose by 1 g/d was also associated with a greater net change Tg of −6.8 mg/dL (95% CI −11.4, −2.2) and each increase in mean baseline Tg level by 1 mg/dL was associated with a greater net change Tg of −0.12 mg/dL (95% CI −0.22, −0.03). However, the few trials that directly compared marine oil doses did not consistently find a dose effect; although, marine oil doses ≥3 g/d all resulted in larger reductions in Tg compared to lower doses, in contrast to doses <3 g/d which had smaller reductions in Tg compared to even lower doses. There were no observational studies evaluating these intermediate CVD outcomes.

In the original report, there was only one RCT of ALA (linseed oil) versus control oil (sunflower seed oil),[17] conducted in the 1960s, that evaluated clinical event outcomes. In this update we identified only one additional RCT of ALA (plant source not reported) versus placebo (oleic acid) in participants with a history of MI that reported clinical outcomes.[18] Given the sparseness of trials of the effect on clinical CVD outcomes of increased ALA intake and the differences between the two trials, no conclusion can be drawn regarding effect of ALA on CVD outcomes. For intermediate outcomes, five ALA RCTs (with doses ranging from 1.4 to 5.9 g/d) evaluated BP outcomes, and four of the five RCTs also evaluated LDL-c, HDL-c, Tg, or Total:HDL-c ratio (2 trials) outcomes. All found no significant differences in these outcomes between ALA and placebo. Thirteen observational studies evaluated ALA intake. The large majority of analyses found no significant associations; only two studies found any significant associations between higher ALA intake and clinical outcomes (reduced all-cause death, SCD, and CHD death risks).

The potential intake threshold-effects of n-3 FA on CVD events could not be determined from the RCTs because there were limited number of RCTs for many outcomes and most RCTs did not find significant effects. Using data from observational studies, the linear dose-response and potential threshold effects of n-3 FA on several CVD events were tested by meta-analytical techniques. There was a near significant association between EPA and DHA intake and CHD across a median dose range of 0.04 to 3.47 g/d (effect size per g/d = 0.90 [95% CI 0.80, 1.01]), and a just-significant association between higher EPA and DHA intake and *higher* risk of ischemic stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 1.03 [95% CI 1.00, 1.07]), but no dose-response relationships found between EPA and DHA intake and hemorrhagic stroke. The interpretations of the threshold-effects (in observational studies) were

limited because differences in associations at lower doses (statistically significant associations between higher intake and lower risk) and associations at higher doses (no significant associations between intake and outcome) were generally similar regardless of the cut point chosen between lower and higher dose analyses.

No differences in effects or associations were found between different populations (healthy or general population, at increased risk for CVD—largely due to dyslipidemia, or with CVD). However, this conclusion is weak given that few studies compared populations, few RCTs were conducted in healthy populations and few observational studies were conducted in at risk or CVD populations.

Limitations

Overall, both RCTs and observational studies (i.e., longitudinal observational and nested case-control studies) included in this systematic review generally had few risk of bias concerns. However, for different analyses, there were some potential applicability issues. For clinical CVD outcomes, all but one of the RCTs was conducted in either high risk individuals or people with existing CVD. In contrast, most observational studies examining the associations between dietary n-3 FA intake or biomarkers of n-3 FA intake and clinical outcomes were conducted in generally healthy populations. Few trials compared n-3 FA dose, formulation, or source. No trial compared different n-3 to n-6 FA ratios of supplements or intake. None of the observational studies attempted to determine a threshold effect of any associations between n-3 FA and the outcome of interest.

There are numerous differences between RCTs and observational studies, making the comparisons across the two study designs difficult to make. Of note, the doses of marine oil supplements (EPA+DHA) in RCTs were often much higher than the highest intake reported for observational studies. Furthermore, not all observational studies explicitly included n-3 FA supplements in their assessment of intake and very few of the RCTs attempted to account for background fish or n-3 FA intake as an effect modifier.

While this report represents a complete systematic review, it does not encompass all trials or longitudinal observational studies that report on CVD and intermediate outcomes. Particularly, if one includes small studies (trials with <30 participants per study group or observational studies with <100 participants, several hundred more studies could potentially have met eligibility criteria. Due to time and resource limitations, we restricted the review to the approximately 100 studies that are most likely to have adequately addressed the primary research questions of interest.

Future research recommendations

Future RCTs should clearly characterize the preparations of n-3 FA, both as individual FA composition and sources of n-3 FA and control oils. It is preferable that standardized n-3 FA oils are analyzed to allow clearer interpretation of what the interventions are and the association between specific n-3 FA and CVD effects. Researchers are encouraged to use standard, common CVD outcomes to allow comparison across studies. Assessment of n-3 FA status and intake should be evaluated at study entry and post-intervention in all study participants with biomarkers and/or food frequency questionnaire to better understand any potentially differential effect of changing n-3 FA intake in populations with different diets (e.g., whether the effect of supplementation differs in people with high- or low-fish diets). If trials include participants with a broad range of n-3 FA status or intake (e.g., with both high- and low-fish diets), subgroup

analyses should be conducted to evaluate possible differential effects based on background diet (or n-3 FA status) The effects (or lack thereof) of marine oils (EPA+DHA) on BP, LDL-c, HDL-c, and Tg are well established so additional RCTs on these intermediate outcomes alone are unlikely to add any new knowledge, and therefore are not needed.

There is an ongoing need to improve self-reported dietary assessment methods and food databases for all nutrients including n-3 FA. As national dietary patterns shift and new processed foods are introduced into the marketplace, food frequency questionnaires need to be updated to ensure accurate estimation of n-3 FA (and other nutrient) intake. Similar to trial registries, a data repository for raw observational study data would greatly improve the transparency of data analyses (potentially reduce both reporting and publication biases) and the appropriateness and methodology of meta-analytical techniques for pooling observational studies. An individual participant-level meta-analysis of observational studies of marine oils could address limitations of the study-level meta-analyses that are currently feasible.

Conclusions

Results from the RCTs of clinical event outcomes are applicable only to at-risk-of-CVD and CVD populations because there is insufficient trial evidence of the effect of n-3 FA on clinical CVD outcomes in healthy populations. Results from the RCTs of intermediate outcomes; however, are applicable to all populations (healthy, at risk, and with CVD) since the trials included a range of people from the different populations.. In contrast, results from observational studies (which did not evaluate intermediate outcomes) are applicable only to generally healthy populations. We graded the strength of the body of evidence for each intervention/exposure and comparison of intervention, and for each outcome by assessing the number of studies, their study designs, the study limitations (i.e., risk of bias and overall methodological quality), the directness of the evidence to the Key Questions, the consistency of study results, the precision of any estimates of effect, the likelihood of reporting bias, and the overall findings across studies. We concluded that there is insufficient evidence regarding the effect of or association between total n-3 FA (ALA + marine oils [EPA+DHA±DPA]) and clinical or intermediate outcomes. There is low strength of evidence of no association between total n-3 FA intake and stroke death, and total MI (each association based on longitudinal observational studies). For marine oil (EPA+DHA±DPA), there is insufficient evidence for most outcomes of interest but there is low to high strength of evidence of a beneficial effect of increased marine oil intake for selected CVD and intermediate outcomes. Specifically, there is high strength of evidence that marine oils clinically and statistically significantly lower Tg—possibly with greater effects with higher doses and in people with higher baseline Tg. There is also high strength of evidence that marine oils statistically, but arguably not clinically, significantly raise HDL-c. There is also high strength of evidence that marine oil significantly lowers Total:HDL-c ratio. There is moderate strength of evidence that marine oil supplementation lowers risk of MACE and CVD death. There is a high strength of evidence of no effect of marine oil on risk of total stroke, but low strength of evidence of no associations of marine oil intake and ischemic or hemorrhagic stroke. There is low strength of evidence for associations between higher EPA+DHA intake and decreased risk of CHD and CHF, based on observational studies. However, there is moderate to high strength of evidence of no effect of (or association between) marine oil and all-cause death, MI, AFib, CHF, sudden cardiac death, revascularization, BP, LDL-c, or LDL:HDL-c ratio. There is also low strength of evidence of no effect of marine oil intake and CHD death.

For individual n-3 FA, there is insufficient evidence regarding the effect of, or association with, EPA, DHA, DPA, SDA, or ALA (specifically) and most CVD clinical outcomes. For EPA, there is low strength of evidence of no association between EPA intake and CHD and between EPA biomarkers and AFib. For DHA, there is moderate strength of evidence of no effect of purified DHA supplementation on BP or LDL-c and low strength of evidence of no association between DHA intake and incident CHD (from observational studies). For DPA (no RCT was identified), there is low strength of evidence of an association between higher DPA biomarker levels and lower risk of AFib. For ALA, there is moderate strength of evidence of no significant effect of ALA intake on BP, LDL-c, HDL-c, or Tg. There is low strength of evidence of no association between ALA intake or biomarker level and CHD or CHD death, AFib, CHF, total and ischemic stroke, based on observational studies.

There is insufficient evidence of direct comparisons between marine oil and ALA intake on CVD outcomes. Across studies, the indirect comparison between marine oil and ALA is unclear, largely because there is insufficient evidence regarding the effect or association of ALA with clinical CVD outcomes. However, where there is high strength of evidence of significant effects of marine oil on improving Tg and HDL-c, there is moderate strength of evidence of no effect of ALA intake on these intermediate outcomes. No RCTs examined the additive effects of n-3 FA versus the effects of individual n-3 FA.

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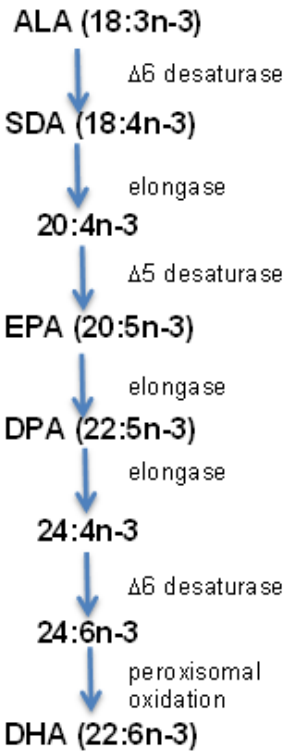
Chapter 1. Introduction

Background

Since the first ecological study published in the late 1970s noted a relatively low cardiovascular mortality in a Greenland Eskimo population with high fish consumption,[1] there have been hundreds of observational studies and clinical trials conducted to evaluate the effect of omega-3 fatty acids (n-3 FA) on cardiovascular disease (CVD) and its risk factors and intermediate markers. The n-3 FA (including alphalinolenic acid [ALA], stearidonic acid [SDA], eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA], and docosahexaenoic acid [DHA]) are a group of essential long-chain and very-long-chain polyunsaturated fatty acids (PUFA) that are involved in the eicosanoid pathway and are incorporated into cell membranes. Eicosanoids (including prostaglandins, thromboxanes, and leukotrienes) have wide ranges of physiologic effects and play a key role in inflammation regulation. The metabolic pathway of n-3 FA is shown in **Figure 1**. ALA is the simplest n-3 FA from which all other n-3 FA are metabolically derived. ALA must come from the diet as it cannot be made by the body. ALA is found in plants, such as leafy green vegetables, nuts, and vegetable oils such as canola, soy, and flaxseed. SDA can be formed from ALA via Δ6 desaturase, the rate-limiting enzyme in the pathway. When SDA enters the metabolic pathway, it is rapidly converted to EPA. EPA can be converted to DPA and vice versa. The conversion rates from ALA to EPA or DHA are highly variable. Good sources of EPA and DHA in the diet include fish, other seafood, other marine sources, and organ meats.

Figure 1. Metabolic pathway of omega-3 fatty acids



Since the publication of the original Agency for Healthcare and Research Quality (AHRQ) n-3 FA systematic reviews in the mid-2000s[2, 3] the topic of n-3 FA and CVD has remained controversial and dynamic. This topic has been evaluated by several expert panels that were considering whether recommendations or reference values for intakes of n-3 FA were warranted, either through naturally occurring sources of n-3 FA (e.g., fish consumption) and/or through the use of dietary supplements and fortified foods.[4-7] In 2002, the Institute of Medicine (IOM) considered the evidence inadequate to establish an estimated average requirement (EAR) for n-3 FA. Thus the IOM established only adequate intake values for ALA, based on current population ALA intake and an apparent absence of deficiency symptoms. For healthy adults, the adequate intake values for ALA are 1.1 g/d for females and 1.6 g/d for males.[5] After evaluating evidence linking the very-long-chain n-3 FA—EPA and DHA—to coronary heart disease and stroke, the IOM panel suggested that n-3 FA may provide beneficial health effects with respect to coronary heart

disease and stroke when consumed at levels ranging from 0.6% to 1.2% of energy (roughly equivalent to 1 to 3 g/d).[5] SDA and DPA have only infrequently been analyzed in regards to their association with CVD. Three other expert reports evaluated the potential health benefits of fish/seafood consumption.[4, 6, 7] Based primarily on the availability of observational study data, these panels consistently suggested that regular consumption of fish and seafood is associated with lower risk of coronary heart disease and cardiac death. These recommendations were based primarily on assumptions of benefits from EPA and DHA and their content in fish and seafood.

Scope and key questions

Scope of the review

The National Institutes of Health's Office of Dietary Supplements (ODS) has a long history of commissioning AHRQ-based systematic reviews and research methodology reports for nutrient-related topics (http://ods.od.nih.gov/Research/Evidence-Based_Review_Program.aspx). n-3 FA and their potential relationship to a broad range of health outcomes formed the basis for nine of these systematic reviews published between 2004 and 2006 and also served as examples for several methodological reports. [8-21]

There are ongoing concerns in the scientific community regarding systematic biases and random errors in the determination of intakes of n-3 FA from dietary and supplement sources using currently available assessment tools. The limitations of the current methods have been discussed elsewhere.[22-24] To date, no alternate methods are available. Until "error-free" or "bias-free" methodologies are developed, it is crucial to evaluate the available data with these methodological quality and limitations in mind. Nutrient biomarkers can provide an objective measure of dietary status. However, the correspondence between intake and biomarker concentration not only reflects recent intake but subsequent metabolism (e.g., elongation, desaturation, metabolism to bioactive compounds). Current biomarkers used to estimate n-3 FA intake include ALA, EPA, DHA, and, less frequently, SDA and DPA, measured in adipose tissue, erythrocytes, plasma, or plasma phospholipids.[25, 26] Adipose tissue FA are thought to reflect long-term intake, erythrocyte FA are thought to reflect the previous 120 day intake, and plasma FA are thought to reflect more immediate intake.[25]

Several recent systematic reviews of randomized controlled trials (RCTs) in individuals with diagnosed CVD or at high risk of CVD have suggested mixed results as to whether there are benefits of very-long-chain PUFA (EPA and DHA) for reducing the risk of adverse cardiovascular outcomes.[19, 27-33] Reasons for the apparent inconsistent scientific conclusions among several of the expert panels and the more recent systematic reviews are varied but may relate, in part, to whether the n-3 FA exposures were from fish (or other marine) or plant sources, or from dietary supplements. The expert reviews also vary as to whether they relied primarily on observational studies or RCTs. [19, 27-33] Studies of different designs each have their own strengths and weakness that may result in differences in conclusions. For example, observational studies based on self-reported dietary assessments (e.g., food frequency questionnaires) may inaccurately estimate n-3 FA intake; RCTs of specific fish or other n-3 FA-rich food may impose an artificial dietary pattern that might not be applicable to the general population; RCTs of supplements might not fully account for differences in background n-3 FA intake; studies using either study design may have subtle differences in eligibility criteria, e.g., length of followup duration, or inclusion of ALA, EPA and DHA or only EPA and DHA, that significantly impacted the final conclusions. Therefore, it is of interest to systematically compare results across different exposure/intervention products and different study types (e.g., interventional vs. prospective

cohort studies), and to account for differences in background n-3 FA intake. Also of interest is a systematic evaluation of possible reasons for inconsistencies between observational and RCT findings,[34] in particular a tabulation of causality-related study features.

The purpose of the current systematic review is twofold: 1) to update earlier reviews of the state-of-the science on the topic of the effects of n-3 FA on CVD,[3] and selected cardiovascular risk factors and intermediate markers of CVD,[2] and 2) to use this new review to collect additional information that would enhance the usefulness of this report for policy and clinical applications. The 2004 reviews screened about 7,500 abstracts and retrieved and screened 768 full text articles for potentially relevant human data. For CVD outcomes, 11 RCTs and one prospective cohort study reported outcomes in individuals with diagnosed CVD, and 22 prospective cohort studies and one RCT reported data on the general population. The report on intermediate CVD outcomes included the 25 largest RCTs with lipid outcomes, an existing systematic review of blood pressure (BP),[35] and six RCTs of BP in people with diabetes (who had been excluded from the existing systematic review). This review updates the previous review for the outcomes included and also expands the scope to include additional CVD outcomes (peripheral vascular disease, congestive heart failure, and arrhythmias); it updates BP and plasma lipid outcomes from, and adds incident hypertension to, the 2004 review of cardiovascular risk factors and intermediate markers of CVD;[2] it adds associations between biomarkers of n-3 FA intake and outcomes.

Key questions

The key questions address both issues of efficacy (i.e., causal relationships from trials) as well as associations (i.e., prospective observational cohort study associations of n-3 FA intake and/or biomarkers with long-term outcomes; biomarker associations reported in RCTs). Compared with the key questions from the 2004 reports, the current key questions expand the scope of the review to include additional cardiovascular outcomes (BP, congestive heart failure, and arrhythmias), focus on the intermediate outcomes plasma lipids and BP, adds the intermediate outcome hypertension, and include associations between biomarkers of intake and outcomes.

4. What is the efficacy or association of n-3 FA (EPA, DHA, EPA+DHA, DPA, SDA, ALA, or total n-3 FA) exposures in reducing CVD outcomes (incident CVD events including all-cause death, CVD death, nonfatal CVD events, new diagnosis of CVD, peripheral vascular disease, congestive heart failure, major arrhythmias, and hypertension diagnosis) and specific CVD risk factors (BP, key plasma lipids)?
    - What is the efficacy or association of n-3 FA in preventing CVD outcomes in people
        - Without known CVD (primary prevention)
        - At high risk for CVD (primary prevention), and
        - With known CVD (secondary prevention)?
    - What is the relative efficacy of different n-3 FA on CVD outcomes and risk factors?
    - Can the CVD outcomes be ordered by strength of intervention effect of n-3 FA?

5. n-3 FA variables and modifiers:
    - How does the efficacy or association of n-3 FA in preventing CVD outcomes and with CVD risk factors differ in subpopulations, including men, premenopausal women, postmenopausal women, and different age or race/ethnicity groups?

- What are the effects of potential confounders or interacting factors—such as plasma lipids, body mass index, BP, diabetes, kidney disease, other nutrients or supplements, and drugs (e.g., statins, aspirin, diabetes drugs, hormone replacement therapy)?
- What is the efficacy or association of different ratios of n-3 FA components in dietary supplements or biomarkers, on CVD outcomes and risk factors?
- How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by ratios of different n-3 FA—DHA, EPA, and ALA, or other n-3 FA?
- How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by source (e.g., fish and seafood, common plant oils (e.g., soybean, canola), fish oil supplements, fungal-algal supplements, flaxseed oil supplements)?
- How does the ratio of n-6 FA to n-3 FA intakes or biomarker concentrations affect the efficacy or association of n-3 FA on CVD outcomes and risk factors?
- Is there a threshold or dose-response relationship between n-3 FA exposures and CVD outcomes and risk factors? Does the study type affect these relationships?
- How does the duration of intervention or exposure influence the effect of n-3 FA on CVD outcomes and risk factors?
- What is the effect of baseline n-3 FA status (intake or biomarkers) on the efficacy of n-3 FA intake or supplementation on CVD outcomes and risk factors?

6. Adverse events:
   - What adverse effects are related to n-3 FA intake or biomarker concentrations (in studies of CVD outcomes and risk factors)?
   - What adverse events are reported specifically among people with CVD or diabetes (in studies of CVD outcomes and risk factors)?

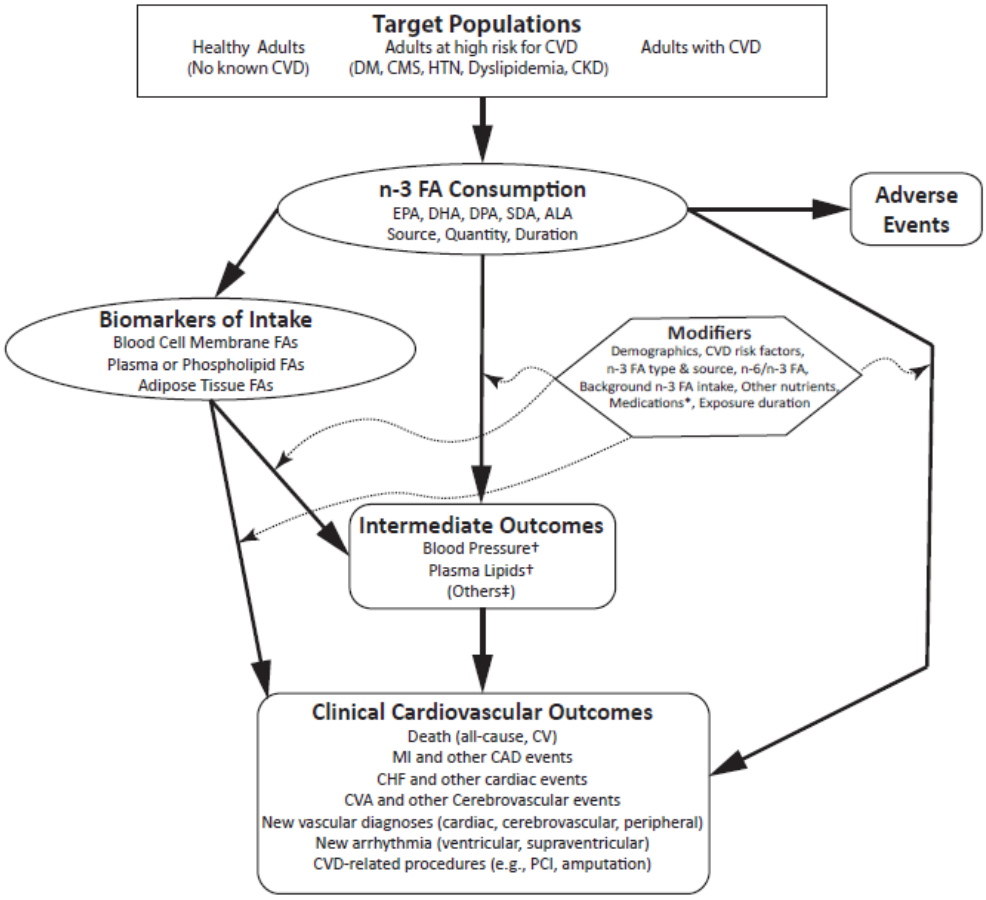
Analytic framework

To guide the assessment of studies that examine the association between n-3 FA intake and cardiovascular outcomes, the analytic framework maps the specific linkages associating the populations of interest, the exposures, modifying factors, and outcomes of interest (**Figure 2**). The framework graphically presents the key components of well-formulated study questions:

1) Who are the participants (i.e., what is the population and setting of interest, including the diseases or conditions of interest)?
2) What are the interventions?
3) What are the outcomes of interest (intermediate and health outcomes)?
4) What study designs are of value?

Specifically, this analytic framework depicts the chain of logic that evidence must support to link the intervention (exposure to n-3 FA) to improved health outcomes.

Figure 2. Analytic framework for omega-3 fatty acid exposure and cardiovascular disease



Legends: This framework concerns the effect of n-3 FA exposure (as a supplement or from food sources) on CVD and cardiovascular risk factors. Populations of interest are noted in the top rectangle, exposure in the oval, outcomes in the rounded rectangles, and effect modifiers in the hexagon.

* Specifically, cardiovascular medications, statins, antihypertensives, diabetes medications, hormone replacement regimens.
† Systolic blood pressure, diastolic blood pressure, mean arterial pressure, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), total/HDL-c ratio, LDL-c/HDL-c ratio, triglycerides.
‡ Many other intermediate outcomes are likely in the causal pathway between n-3 FA intake and cardiovascular outcome, but only blood pressure and plasma lipids are included in the review.

ALA = alphalinolenic acid, CAD = coronary artery disease, CHF = congestive heart failure, CKD = nondialysis-dependent chronic kidney disease, CMS = cardiometabolic syndrome, CVA = cerebrovascular accident (stroke), CVD = cardiovascular disease, DHA = docosahexaenoic acid, DM = diabetes mellitus, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FA = fatty acid, HTN = hypertension, MI = myocardial infarction, n-3 = omega-3, n-6 = omega-6, PCI = percutaneous coronary intervention, SDA = stearidonic acid.

Chapter 2. Methods

The present review evaluates the effects of, and the associations between, n-3 FA (EPA, DPA, ALA and n-3 FA biomarkers) and CVD outcomes. The Brown Evidence-based Practice Center (EPC) conducted the review based on a systematic review of the published scientific literature using established methodologies as outlined in the AHRQ Methods Guide for Effectiveness and Comparative Effectiveness Reviews.[36]

The review is conducted in parallel with a systematic review of n-3 FA and child and maternal health, conducted by another EPC. Several aspects of the review are being coordinated, including eligibility criteria and search strategies regarding interventions and exposures structure of the reviews, and assessments of the studies' risk of bias, strength of the bodies of evidence, and extraction of study characteristics needed to assess causality.

Topic refinement and review protocol

We convened a Technical Expert Panel (TEP) to help refine the research questions and protocol. The TEP included five experts in nutrition, n-3 FA research specifically, CVD epidemiology, and cardiology. Also included in the discussions with the TEP were the Director of and a Senior Scientist at the Office of Dietary Supplements (ODS), and the AHRQ Task Order Officer. We discussed the key questions, analytic framework, study eligibility criteria, literature search, and analysis plans.

In regards to the populations of interest, we explicitly expanded the definition of the at risk for CVD population to include adults with cardiometabolic syndrome (and related conditions) and nondialysis dependent chronic kidney disease. Regarding the interventions of interest, we discussed the changes from the original AHRQ reports on n-3 FA, specifically that we included only studies that quantify n-3 FA content of the intervention, and that we added n-3 FA biomarkers as an exposure of interest. We also clarified that we excluded weight loss interventions that included n-3 FAs as part of the intervention. Regarding outcomes of interest, we refined the list of "major lipids" of interest to include only LDL-c, HDL-c, triglycerides, LDL-c to HDL-c ratio, and total cholesterol to HDL-c ratio. Compared to the original n-3 FA and CVD outcome report, we added peripheral vascular disease, arrhythmia events, congestive heart failure, and incident hypertension. We discussed a number of potential modifiers of interest to be searched for, including demographic features, weight, BP, source and type of n-3 FA, exposure duration, C reactive protein level, and specific co-interventions (i.e., statins, vitamin E).

It was agreed to maintain a minimum duration of followup of 1 month for intermediate outcomes (lipids and BP) and 1 year for all clinical outcomes. We agreed to include only RCTs of specific comparisons of interventions and large, prospective, longitudinal observational studies of exposure (either baseline dietary intake or biomarker level). We also agreed to include the RCTs that are largest or report subgroup or factorial analyses, and the largest observational studies to constrain the total number of included studies to approximately 75 to 100. The search strategy was refined based on suggestions from the TEP. The TEP agreed that the primary literature search would be conducted for the period from 2002 to the present to capture studies published since the original EPC report, with older studies to come from existing systematic reviews including the original EPC report. For new topics (e.g., biomarkers, peripheral vascular disease), the TEP agreed that searches back to 2000 would be sufficient to capture relevant analyses.

In addition, in separate discussions with the ODS representative and our TOO we considered how and whether to assess the concept of causality, particularly for the observational

studies. After discussion of the Bradford Hill criteria and related issues regarding causality,[37] we agreed upon the creation of an appendix table (Appendix G) that provides the study-level data for items that may be pertinent for users of this report to assess causality.

Furthermore, we had joint discussions with the Southern California EPC—which conducted a parallel report of n-3 FA and maternal and child health—and our TOO and the ODS representative to coordinate our protocols and processes. The protocol was entered into the PROSPERO register (registry number CRD42014015602).

Literature search

Search strategy

We conducted literature searches of studies in MEDLINE, both the Cochrane Central Trials Registry and Cochrane Database of Systematic Reviews, EMBASE, and CAB Abstracts from 2002 to [19 November 2014] (to overlap with the last search run for the 2004 reviews). We searched earlier publications back to 2000 for the newly added outcomes (peripheral vascular disease, congestive heart failure, arrhythmias, hypertension) and for biomarkers of n-3 FA intake. We also included all studies from the original reviews that continued to meet eligibility criteria. We revised the search strategy used in the original reviews to capture new terms for n-3 FA, biomarkers, and additional outcomes. In electronic searches, we combined terms for n-3 FA (and biomarkers), CVD and risk factors (BP, plasma lipids, hypertension), limited to humans, English language, and relevant research designs. Titles and abstracts were screened to identify articles relevant to each Key Questions. We also reviewed reference lists of related systematic reviews. We invited TEP members to provide additional citations. In addition, a call for potentially relevant articles was posted on the Federal Register (in lieu of Scientific Information Packets). [The search will be updated upon submission of the draft report for peer and public review.] **Appendix A** displays the current complete search strategy.

Inclusion and exclusion criteria

The current eligibility criteria are mostly similar to the criteria used in the original 2004 review. The populations remain the same. The interventions and exposures have been expanded to include n-3 FA biomarkers. The list of CVD outcomes of interest has been expanded. Similar study designs are included.

For all key questions, the eligibility criteria are:

Populations

- Healthy adults (≥18 years) without CVD or with low to intermediate risk for CVD
- Adults at high risk for CVD (e.g., with diabetes, cardiometabolic syndrome, hypertension, dyslipidemia, nondialysis dependent chronic kidney disease)
- Adults with clinical CVD (e.g., history of myocardial infarction, angina, stroke, arrhythmia)
- Exclude populations chosen for having a non-CVD or non-diabetes-related disease (e.g., cancer, gastrointestinal disease, rheumatic disease, dialysis)

Interventions/Exposures

- n-3 FA supplements
- n-3 FA supplemented foods (e.g., eggs)

- n-3 FA content in diet (e.g., from food frequency questionnaires)
- Biomarkers of n-3 FA intake

- n-3 FA content of food or supplements must be explicitly quantified. Therefore, studies such as those of fish diet where only servings per week are defined or Mediterranean diet studies without n-3 FA quantified are excluded. The n-3 FA quantification can be of total n-3 FA, of a specific n-3 FA (e.g., ALA) or of combined EPA+DHA ("marine oil").
- Exclude mixed interventions of n-3 FA and other dietary or supplement differences (e.g., n-3 FA and vitamin E versus placebo; n-3 FA as part of a low fat diet versus usual diet). However, factorial design (and other) studies that compare (for example) n-3 FA versus control, with or without another intervention (e.g., statins) are included.
- Exclude n-3 FA dose ≥6 g/day, per the original review's protocol based on the assessment that n-3 FA intake above this amount is impractical and has little relevance on health care recommendations.
- Exclude weight loss interventions

Comparators
- Placebo or no n-3 FA intervention
- Different n-3 FA source intervention
- Different n-3 FA concentration intervention
- Different n-3 FA dietary exposure (e.g., comparison of quantiles)
- Different n-3 FA biomarker levels (e.g., comparison of quantiles)

Outcomes
- All-cause death
- Cardiovascular, cerebrovascular, and peripheral vascular events:
    - Fatal vascular events (e.g., due to myocardial infarction, stroke)
    - Total incident vascular events (e.g., myocardial infarction, stroke, transient ischemic attack, unstable angina, major adverse cardiovascular events [MACE]; total events include fatal and nonfatal events; total stroke includes ischemic and hemorrhagic stroke)
    - Coronary heart disease, new diagnosis
    - Congestive heart failure, new diagnosis
    - Cerebrovascular disease, new diagnosis
    - Peripheral vascular disease, new diagnosis
    - Ventricular arrhythmia, new diagnosis, including sudden cardiac death [SCD]
    - Supraventricular arrhythmia, new diagnosis
    - Major vascular interventions/procedures (e.g., revascularization, thrombolysis, lower extremity amputation, defibrillator placement)
- Major CVD risk factors (intermediate outcomes):
    - BP (new-onset hypertension, systolic, diastolic, and mean arterial pressure)
    - Key plasma lipids (i.e., high density lipoprotein cholesterol [HDL-c], low density lipoprotein cholesterol [LDL-c], total/HDL-c ratio, LDL-c/HDL-c ratio, triglycerides)
- Adverse events (e.g., bleeding, major gastrointestinal disturbance), only from intervention studies of supplements

Timing

- Clinical outcomes, including new-onset hypertension (all study designs): ≥1 year followup (and intervention duration, as applicable)
- Intermediate outcomes (BP and plasma lipids) (all study designs): ≥1 month followup
- Adverse events (all study designs): no minimum followup

Setting

- Community-dwelling (noninstitutionalized) individuals

Study Design

- RCTs (all outcomes)
- Randomized cross-over studies (BP and plasma lipids, adverse events), minimum washout period to be determined
- Prospective nonrandomized comparative studies (clinical outcomes, adverse events)
- Prospective cohort (single group) studies, where groups are compared based on n-3 FA intake or intake biomarker values (clinical outcomes)
- Exclude: Retrospective or case control studies or cross-sectional studies (but include prospective nested case control studies). Studies must have measure of intake prior to outcome.

- Minimum sample sizes
    - RCTs
        - We aimed for a minimum of about 25 RCTs for each of the BP and plasma lipid outcomes. We preferentially included RCTs that reported relevant subgroup, interaction, or factorial analyses.
            - For RCTs with BP or lipid outcomes with subgroup, interaction, or factorial analyses, we included parallel design RCTs with a minimum of 30 participants per arm, factorial RCTs with a minimum of 30 participants per n-3 FA intervention, and crossover trials with a minimum of 20 participants.
            - For RCTs with lipid outcomes without subgroup analyses, we included parallel design RCTs with a minimum of 200 participants per arm, factorial RCTs with a minimum of 200 participants per n-3 FA intervention, and crossover trials with a minimum of 100 participants.
            - For RCTs with BP outcomes without subgroup analyses, if followup was ≥6 months, we included all RCTs; if followup was <6 months (≥1 month), we included parallel design RCTs with a minimum of 80 participants per arm, factorial RCTs with a minimum of 80 participants per n-3 FA intervention, and crossover trials with a minimum of 40 participants.
            - For RCTs with CVD event outcomes, we included all RCTs with at least 10 participants per arm.
    - Longitudinal observational studies
        - We aimed for a minimum of about 10 observational studies for each broad clinical outcome (see bullets below) and also for dietary marine oils, dietary ALA, marine oil biomarkers, and ALA biomarkers.

- For cardiac event outcomes, we included observational studies with at least 10,000 participants.
- For death outcomes, we included observational studies with at least 10,000 participants.
- For stroke event outcomes, we included observational studies with at least 3000 participants.
- For stroke event outcomes, we included observational studies with at least 3000 participants.
- For arrhythmia event outcomes, we included observational studies with at least 2000 participants.
- For congestive heart failure event outcomes, we included observational studies with at least 700 participants.
- For peripheral vascular disease event, incident hypertension, MACE, and revascularization outcomes, we included observational studies with at least 500 participants.
- We screened smaller sample size observational studies (starting with the largest studies) to include additional studies of ALA biomarkers, regardless of the outcomes analyzed.

  - In all instances, if a study met eligibility criteria for any outcome, we extracted all outcomes of interest from that study; therefore, there are multiple instances of studies being included for an outcome even though the study might not have met study size criteria for that specific outcome.

- English language publications
- Peer reviewed publications

Study selection

All citations found by literature searches or through other sources were independently screened by two researchers. Upon the start of citation screening, we implemented a training session where all researchers screen the same articles and conflicts were discussed. We iteratively continue training until we have reached agreement regarding the nuances of the eligibility criteria for screening. During double-screening, we resolved conflicts as a group. All screening of literature citations was done in the open-source, online software Abstrackr (http://abstrackr.cebm.brown.edu/).

All potentially eligible abstracts were entered into an "evidence map". From each abstract, a single researcher extracted data on the study sample size (total), study design, study duration, the population category (healthy, at risk, CVD), the specific n-3 FA analyzed, whether biomarkers were reported, whether subgroup or factorial analyses were reported, and the outcomes mentioned in the abstract.

Based on the study descriptions in the evidence map, we selected the largest studies and those with subgroup or factorial analyses for full text review, with the goals of including a minimum of about 25 RCTs for each of the BP and plasma lipid outcomes, all RCTs with clinical outcomes, and a minimum of about 10 observational studies for each broad clinical outcome and also for dietary marine oils, dietary ALA, marine oil biomarkers, and ALA biomarkers.

Data extraction

Each study was extracted by one methodologist. The extraction was reviewed and confirmed by at least one other experienced methodologist. Disagreements were resolved by discussion among the team, with the team leader, or between extractors. Data were extracted into customized forms in Systematic Review Data Repository (SRDR) online system (http://srdr.ahrq.gov) and Excel spreadsheets, each designed to capture all elements relevant to the Key Questions. Upon completion of the review, the Excel spreadsheets (of observational study results data) [will be] uploaded into SRDR and the database [will be] made accessible to the general public (with capacity to read, download, and comment on data). The basic elements and design of these forms include elements that address population characteristics; descriptions of the interventions, exposures, or biomarker status (and comparators) analyzed; outcome definitions; enrolled and analyzed sample sizes; study design features; results; and risk of bias assessment. The form was developed off the forms used for the original review. We also included questions pertinent to issues related to causality. We tested the forms on several studies and revised them as necessary before full data extraction.

Quality (risk of bias) assessment of individual studies

We assessed the methodological quality of each study based on predefined criteria. For RCTs, we used the Cochrane risk of bias tool,[38] which asks about risk of selection bias, performance bias, detection bias, attrition bias, reporting bias, and other potential biases. For observational studies, we used relevant questions from the Newcastle Ottawa Scale.[39] Additionally we included nutrition study specific risk of bias questions (e.g., related to uncertainty of dietary assessment measurements.[11, 13, 40] Any quality issues pertinent to specific outcomes within a study were noted and applied to those outcomes. Any quality issues pertinent to specific outcomes within a study were noted and considered when determining the overall strength of evidence for conclusions related to those outcomes.

Data synthesis

All included studies were summarized in narrative form and in summary tables that tabulate the important features of the study populations, design, intervention, outcomes, and results. Other study data are in Appendix tables.

We analyzed different study designs separately and compared and contrasted populations, exposures, and results across study designs. We examined any differences in findings between observational and intervention studies, and evaluated the risk of bias factors as possible explanations for any heterogeneity.

Statistical analyses were conducted in Stata version 13.1 (StataCorp, College Station, Texas). We conducted random effects model meta-analyses of comparative studies (RCTs) if, for each set of studies with the same outcome and intervention and comparator pair, there were at least six studies. We used the restricted maximum likelihood method (with the metareg command) to calculate the overall and population-specific (healthy, at risk, CVD) effect sizes. For trials that compared multiple n-3 FA doses to placebo, we included only the comparison of the highest dose of n-3 FA versus placebo in meta-analysis. Likewise, for trials that compared both purified EPA and DHA to placebo, we arbitrarily included only the EPA versus placebo comparison.

We summarized included observational studies both qualitatively and quantitatively. We looked at hazard ratios (HR) and their respective confidence intervals of categorical outcomes of interest for each quantile of omega-3 exposure (intake or biomarker level) within a study versus

its reference quantile. The HRs were plotted at the median dose with in a quantile's dose range (see below). Separate graphs were drawn for each combination of specific n-3 FA, measure type (e.g., intake, phospholipid level, percent FA), and outcome. We combined analyses of EPA+DHA and DPA+DHA+DPA. Within each graph, we plotted each reported cohort (i.e., from a given study, we plotted the analysis of the total cohort if that was reported, or we plotted both subgroup analyses—usually men and women—if only those were reported). We use unique symbols across graphs for all adults, men, women, and other subgroups.

When a study did not report the median doses for specific dose quantiles, we estimated them using the following rules. If the study provided the minimum and maximum dose within a quantile, we used the midpoint as the median dose. For the lowest and highest quantiles, if only one end of the range was reported (e.g., lowest quintile was <0.5 g/d), we estimated the median dose to be 20% less (or more) than that quantile's upper (or lower) range.[41] For studies that did not report the number of participants or person-years per quantile, we equally divided the total for the whole cohort to estimate the numbers per quantile.

We meta-analyzed observational cohorts when at least four cohorts analyzed the same n-3 FA, measure, and outcome. For each study cohort to be meta-analyzed, we used the STATA glst command to retrieve a set of coefficients and covariance matrices from generalized least squares trend estimation of splines with one knot each (exposure dose where the curve slope is allowed to change) across a range of knot points. Separately for ALA intake and EPA+DHA±DPA intake (the n-3 FA measured that had sufficient data for meta-analysis), we determined the range of knots for spline models by ordering the median values of all quantiles of all ALA or all EPA+DHA±DPA intake analyses being meta-analyzed (across outcomes) and selected a range from approximately the 5th lowest to 5th highest median values. Knot points were rounded to the nearest 0.1 g/d and stepped up in 0.1 g/d units to the highest knot point. We used the STATA glst command (generalized least squares) to estimate the splines for each cohort being meta-analyzed, across the range of knots. For a particular cohort, if a knot fell outside the cohort's n-3 FA dose range, we generated a linear model without a knot. We then used the STATA mvmeta command to meta-analyze these spline models (at each knot). We captured the Akaike information criterion (AIC) for each meta-analyzed spline (at each knot). We tabulated all meta-analyzed spline models for each set of studies (within a range of knots that pertain to each set of studies). In the figures of the association of n-3 FA exposure versus risk of outcome, we included the meta-analysis spline with the best fit (the lowest AIC value).

Summary of causality-related study features

We compiled a pair of appendix tables (Appendix G) with data related to possible causality criteria. The list of items in this table was compiled based on discussions between the EPCs and ODS after discussion of the Bradford Hill criteria [34] and other issues related to determining causality. The table includes a listing of included studies with their population category (healthy, at high CVD risk, with CVD), CVD risk type (e.g., diabetes, hypertension, chronic kidney disease, dyslipidemia), demographics (age, sex, race), cardiovascular history, cardiovascular risk factors (BP, plasma lipids, weight), baseline n-3 FA intake, n-3 FA source, n-3 FA type, how n-3 FA intake measured, study design (e.g., RCT, prospective or retrospective longitudinal cohort, or other design), exposure duration, followup duration, outcomes reported, effect sizes, difference in n-3 FA intake (between low and high intake groups), and a dose-corrected effect size.

Strength of the body of evidence

We graded the strength of the body of evidence as per the AHRQ Methods Guide on assessing the strength of evidence for each outcome.[42] Following the standard AHRQ approach, for each intervention and comparison of intervention, and for each outcome, we assessed the number of studies, their study designs, the study limitations (i.e., risk of bias and overall methodological quality), the directness of the evidence to the Key Questions, the consistency of study results, the precision of any estimates of effect, the likelihood of reporting bias, and the overall findings across studies. Based on these assessments, we assigned the strength of evidence rating as being either high, moderate, or low, or there being insufficient evidence to estimate an effect. For outcomes with ≤2 RCTs providing evidence, the highest possible strength of evidence was "Low" under the presumption that observational studies (that analyzed the association between a one-time estimate of n-3 FA status and clinical outcomes ≥1 year in the future) cannot alone provide good evidence of an effect of n-3 FA intake. For outcomes with ≤2 RCTs, ≤2 observational studies of intake, and ≤2 observational studies of biomarkers, the strength of evidence grade was "Insufficient." If we were unable to conclude a finding of an association or effect, or no association or effect, (generally because of imprecision or inconsistency across studies), we determined that the evidence was "Insufficient" since it is not meaningful to state that there is a low strength of evidence of an unclear effect/association.

The strength-of-evidence dimensional rating are summarized in Evidence Profile tables detailing our reasoning for arriving at the overall strength of evidence rating. Study characteristics related to causality are tabulated in Appendix G.

Applicability

We qualitatively assessed the applicability within and across studies with reference to whether people in the studies are in the three populations of interest (healthy, at risk, and with CVD), and as pertains to n-3 FA source, type, and dose/exposure.

Peer review and public commentary

A draft version of this report [is being] reviewed by a panel of expert reviewers, including representatives from [pending] and the general public. The reviewers included experts in [pending]. These experts were either directly invited by the EPC or offered comments through a public review process. Revisions of the draft [will be] made, where appropriate, based on their comments. The draft and final reports [will] also reviewed by the Task Order Officer and an Associate Editor from another EPC. However, the findings and conclusions are those of the authors, who are responsible for the contents of the report.

Chapter 3. Results

The Results chapter is organized as follows. The chapter starts with an overall description of the included studies and their risk of bias assessment. The bulk of the chapter is organized by outcome, with a description first of the RCTs and their subgroup analyses, followed by the observational studies and their subgroup analyses. Within each description of studies, we follow the basic pattern of first describing the evidence regarding total n-3 FA combined, then ALA, the individual long-chain n-3 FA (EPA, DHA, DPA, SDA), and then combined long-chain n-3 FA (EPA+DHA±DPA). Within the description of the observational studies, we first present the results of associations with n-3 FA intake followed by n-3 FA biomarkers.

Appendix A presents the literature search strategies. **Appendix B** lists the articles that were reviewed in full text that were excluded, with their rejection reasons. **Appendix C** presents the study-level risk of bias assessments of all studies. **Appendix D** presents study-level baseline data. **Appendix E** presents study-level design features. **Appendix F** presents the study-level results data for the observational studies. **Appendix G** presents the "causality tables" described in the Methods section.

Summary of studies

The literature searches yielded 9676 citations (**Figure 3**). Reference lists from existing systematic reviews yielded 203 additional citations (which mostly represented articles published before 2002). Of these, 758 abstracts met basic eligibility criteria. As described in the Methods chapter (under *Study selection*), using an evidence map process, we selected 421 articles for full text review, of which 144 articles met eligibility criteria, representing 55 RCTs (in 85 articles) and 33 longitudinal observational studies (in 59 articles).[43-188]

Study risk of bias

Across RCTs, the studies generally had few risk of bias concerns (**Figure 4, Appendix C**). Sixteen of 55 RCTs (29%) had no risk of bias / study quality limitations; an additional 30 RCTs (55%) had one risk of bias limitation. None of the remaining 9 RCTs (16%) had more than four study limitations (of 10 explicitly assessed potential limitations). The most common risk of bias limitation was a lack of intention-to-treat analyses; 14 RCTs (25%) clearly did not conduct intention-to-treat analyses (one of these conducted an intention-to-treat analysis for the outcome death, but not for lipid outcomes); six additional RCTs (11%) were unclear whether intention-to-treat analyses were conducted. Ten RCTs (18%) did not blind study participants (and three additional, 5%, were unclear whether they blinded participants), often because the intervention was dietary and could not be blinded. However, only four RCTs (7%) clearly did not blind outcome assessors (nine additional RCTs, 16%, were unclear regarding outcome assessor blinding). Attrition bias, primarily due to dropout rates greater than 20 percent, was present in 8 RCTs (15%). Other potential biases were less common. A single study had four high risk of bias issues (poor allocation concealment, unblinded participants, unblinded outcome assessors, and likely reporting bias).[62] Three RCTs had three high risk of bias issues each (two studies each with unblinded participants, possible reporting bias, lack of intention-to-treat analyses; one study each with unblinded outcome assessors, attrition bias, and differences in compliance across groups).[51, 154, 167]

Figure 3. Literature flow

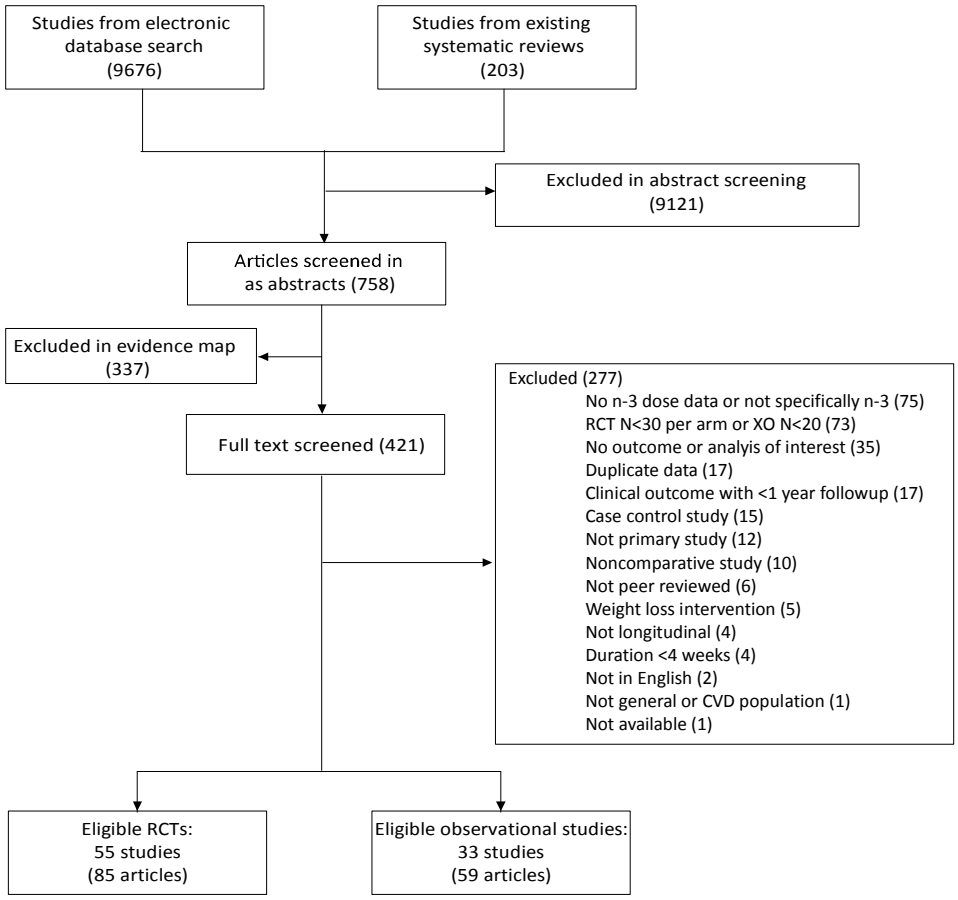
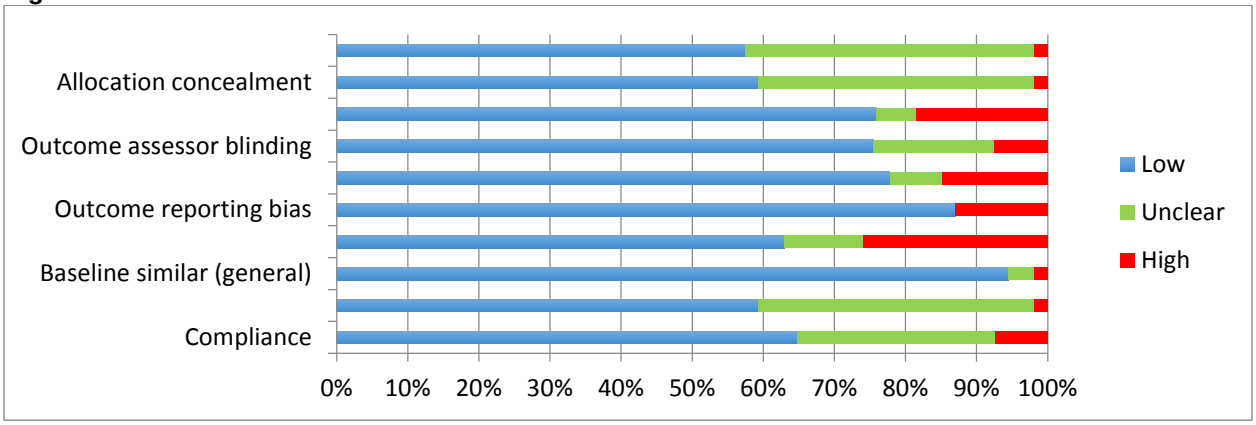


Figure 4. Risk of bias of randomized controlled trials



Across the observational studies, there were fairly few risk of bias concerns (**Figure 5**). No study was deemed to have high risk of selection bias (regarding whether the outcome was present at baseline), but for three of 33 studies (9%) it was unclear. Two studies (6%) did not

adjust analyses for confounders or other factors. Three studies (9%) did not blind outcome assessors and for another three studies (9%) it was unclear whether they were blinded. Incomplete outcome data analysis was of concern in only one study (3%), but was unclear in another four studies (12%). In three of 26 studies (12%) there was inadequate reporting of the dietary assessment instrument, but only six studies (23%) explicitly estimated n-3 FA from both dietary and supplement sources. The most frequent reporting inadequacy related to whether the ranges and distribution of n-3 FA exposures were fully reported; 15 of 33 studies (45%) did not fully report such data. Only five of 33 studies (15%) had two study limitations (of six explicitly assessed).[75, 102, 164, 177, 186]

Figure 5. Risk of bias of longitudinal, prospective observational studies

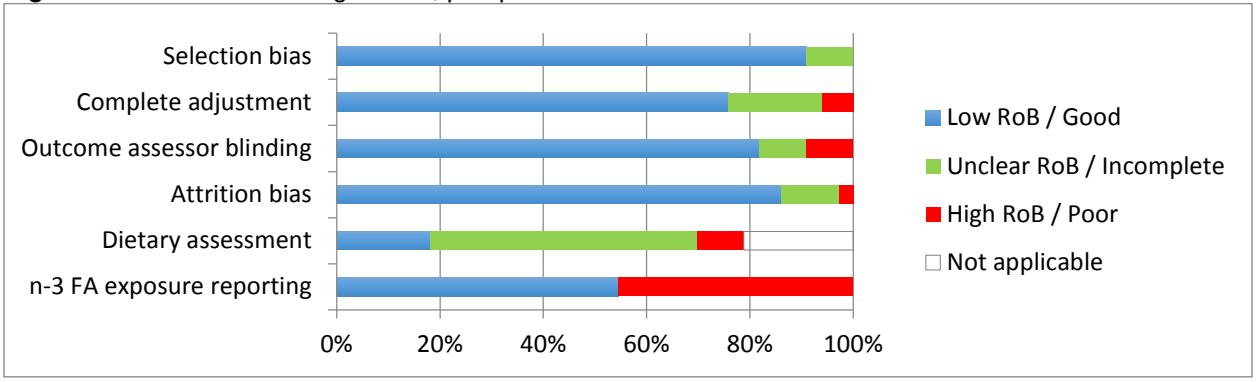


Table X enumerates studies by n-3 FA, strength of evidence, and overall effect or association by outcome. The table highlights the lack of sufficient evidence for most clinical CVD outcomes (empty cells and unshaded cells with black font). Only for marine oil (EPA+DHA) is there sufficient evidence for beneficial effect (or association) of higher n-3 FA intake. The body of evidence provides no sufficient evidence of a significant effect (or association) of ALA on CVD outcomes or examined risk factors.

Table X. Enumeration of studies by outcome and n-3 FA

Outcome	Total n-3 FA			Marine oil			EPA			DHA			DPA			ALA			MvA
	R	OI	OB	R	OI	OB	R	OI	OB	R	OI	OB	R	OI	OB	R	OI	OB	R
Total		7	3	47	21	5	4	8	9	3	8	10		2	6	6	12	7	3
ACS		1			1	1		1	1		1	1			1				
Ang, Stable																1			
Ang, Unstable				2			1												
AFib			1	3	3							1			3		3		
Card Death				4	1	1			1			1			1			1	
CVD																			
CVD Death		3	1	6	4			2	1		2	1			1	1	2	2	
CHF			1	3	5	2			3			3			1	1	4	3	
CHF Death		1		1															
CHD			2		7	1		2	3		2	3			3		6		
CHD Death		2	1	3	7		1	2	1		2	1			1	1	4	1	
Death, All		1	1	15	3				3			3			1	1		2	
HTN		2			1														
MACE		3		8	3	2	1	1	3		1	4			3	1	2		
MI		3		7	1			1			1					1			
MI Death		2			1														
Revasc				5	1		1												
CVA Dth, Hem					1			1			1								
CVA Dth, Isch		1			1			1			1								
CVA Dth, Tot		3	1	2	1		1	1	1		1	1			1				
CVA, Hem			1		4	1			1			1			1		2	2	
CVA, Isch			1		4	2		1	2		1	2			1		2	3	
CVA, Tot		1	1	6	4	2			1			1			1	1	3	2	
SCD		1	1	8	1				1										
Vent Arrh																1			

Outcome	Total n-3 FA			Marine oil			EPA			DHA			DPA			ALA			MvA
	R	OI	OB	R	OI	OB	R	OI	OB	R	OI	OB	R	OI	OB	R	OI	OB	R
SBP	1			22			2			3						4			
DBP	1			22			2			3						4			
MAP				3			2												
LDL-c	2			33			2			3						4			
HDL-c	2			33			2			3						4			
Tg	2			34			2			2						4			
LDL:HDL-c				3															
Total:HDL-c	2			7			1			1						2			

Table summarizing the number of studies that report on each evaluation of a type of omega-3 fatty acid (n-3 FA) and outcome, by study design. Green font and shading indicate high strength of evidence for the given n-3 FA and outcome pair. Orange font and shading indicate moderate strength of evidence. Red font and shading indicate low strength of evidence. Colored shading indicates evidence of a significant effect or association between higher n-3 FA intake/level and a reduced risk of the outcome or status of the intermediate outcome. Colored fonts indicate evidence of no significant effect or association of the n-3 FA on the outcome. Black, unshaded font indicates insufficient evidence.

Abbreviations: ACS = acute coronary syndrome; AFib = atrial fibrillation; ALA = alphalinolenic acid; Ang = angina; Card = cardiac; CHD = coronary heart disease; CHF = congestive heart failure; CVA = cerebrovascular accident (stroke); CVA Dth = stroke death; CVA, Hem = hemorrhagic stroke; CVA, Isch = ischemic stroke; CVA, Tot = total stroke; CVD = cardiovascular disease; DBP = diastolic blood pressure; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; HDL-c = high density lipoprotein cholesterol; HTN = incident hypertension; LDL:HDL-c = LDL-c to HDL-c ratio; LDL-c = low density lipoprotein cholesterol; MACE = major adverse cardiac event; MAP = mean arterial pressure; MI = myocardial infarction; MvA = direct comparison of marine oil and ALA (in randomized controlled trials); n-3 FA = omega-3 fatty acids; OB = observational studies of n-3 FA biomarkers; OI = observational studies of n-3 FA intake; R = randomized controlled trials; Rd = randomized controlled trials with dose comparison; SBP = systolic blood pressure; SCD = sudden cardiac death; Tg, = triglycerides; Total:HDL-c = total cholesterol to HDL-c ratio; Vent Arrh = ventricular arrhythmia.

Major Adverse Cardiovascular Events (MACE)

Randomized Controlled Trials

Eight RCTs reported the composite outcome MACE (**Table A.1**).[56, 78, 88, 114, 126, 153, 168, 187] Of these, three studies were conducted in a total of 31,713 people at risk of CVD including dyslipidemia,[78, 187] or a combination of various risk factors.[153] Five studies were conducted in a total of 27,096 people with CVD, defined as a history of CVD,[88] a history of MI,[114] persistent AFib,[123] heart failure,[168] or, in one study, either a history of CVD or of diabetes.[56] None of the RCTs were conducted in a generally healthy population.

Marine oil vs. placebo

Meta-analysis of the eight RCTs of marine oil versus placebo yielded a just-significant summary effect size for risk of MACE: HR=0.95 (95% CI 0.90, 1.00; P=0.047) (**Figure A.2**).[56, 78, 88, 114, 126, 153, 168, 187]

At risk for CVD population

Among people at risk of CVD, one trial compared EPA ethyl ester combined with statin with control (statin alone) in 18,645 participants with dyslipidemia (19.5% with CAD) [187] and two studies compared marine oil (EPA+DHA) to placebo (olive oil or corn oil) in a total of 13,068 participants with dyslipidemia or multiple CVD risk factors.[78, 153] In the study of EPA ethyl ester, the dose of EPA was 1.8 g/d; in the other two studies the doses of EPA+DHA were 0.85 and 2.02 g/d with EPA to DHA ratio either 0.9 or 1.5. Compliance was monitored and the adherence level was greater than 90 percent in one study,[78] but not reported in the other two studies. The duration of followup ranged from 3 to 5 years.

In one RCT, EPA supplementation (1.8 g/d) had a significant additive effect (to statin therapy) on reducing the risk of MACE (including sudden cardiac death, fatal and nonfatal MI, and nonfatal unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting) compared with statin alone after 5 years of followup (HR 0.81, 95% CI 0.69, 0.95).[187] The other two trials found that EPA+DHA supplementation (0.85 and 2.02 g/d) did not significantly reduce the risk of MACE (heterogeneous definitions) compared with placebo (HR 0.98, 95% CI 0.88, 1.08; HR 0.89, 95% CI 0.55, 1.44)

Subgroup meta-analysis yielded a summary HR of 0.96 (95% CI 0.91, 1.02).

CVD population

Among people with CVD, five RCTs (four parallel design, one a 2-by-2 factorial RCT) evaluated MACE. The four simple RCTs compared marine oil (EPA+DHA) to placebo (olive oil in two studies and sources not reported in the other two studies) in a total of 22,259 participants with DM and history of CVD, all CVD, heart failure or previous persistent AFib.[56, 88, 123, 168] The 2-by-2 factorial RCT that compared the effects of a margarine supplemented with EPA+DHA alone (0.4 g/d), a combination of both EPA+DHA and ALA margarines, and ALA alone (2 g/d) with placebo margarine (oleic acid) in 4837 participants with a history of MI.[114] (The 2-by-2 factorial trial reported only analyses of EPA+DHA vs. placebo and ALA vs. placebo.)

Among the five trials that compared marine oil (EPA+DHA) to placebo, the doses of EPA+DHA used ranged from 0.4 to 0.882 g/d, and the EPA to DHA ratio ranged from 0.5 to 2. Reported in four studies, the compliance ranged from 70 to 90 percent. The duration of followup

ranged from 1 to >6 years. Four of the five trials found that EPA+DHA supplementation did not significantly reduce the risk of MACE (heterogeneous definitions) compared with placebo (HR ranging from 0.88 to 1.08).[56, 88, 114, 123] The fifth trial found that EPA+DHA supplementation significantly reduced the risk of MACE (defined as death from any cause or admission to the hospital for cardiovascular reasons) compared with placebo in 6975 participants with heart failure (HR 0.92, 95% CI 0.85, 0.99).[168]

Subgroup meta-analysis yielded a summary HR of 0.90 (0.78, 1.05).

ALA vs. placebo

CVD population

In the 2-by-2 factorial RCT, the groups that received ALA margarines had no significant difference in the risk of MACE compared with placebo margarines (HR 0.92; 95% CI 0.73, 1.11).[114]

RCT subgroup analyses

Three RCTs reported subgroup analysis for MACE (**Table A.2**). In one trial, EPA+DHA (vs. placebo) lowered the risk of MACE in women (HR=0.82) in contrast with the effect in men (HR 1.04) and the difference between women and men was statistically significant (P interaction 0.04).[153] The second trial found no difference in effect of EPA versus placebo between men and women (HR 0.76 vs 0.87, P-interaction 0.43).[187] This study analyzed several other subgroups, but found no significant differences in effect between any subgroups. These included age ≥61 vs. <61 years, BMI ≥24 vs. <24 kg/m^2, triglycerides ≥270 vs. <270 mg/dL, triglycerides ≥150 vs. <150 mg/dL, HDL-c ≥58 vs. <58 mg/dL, LDL-c ≥181 vs. <181 mg/dL, history of CAD vs. no CAD, smoker vs. nonsmoker, diabetes vs. no diabetes, and HTN vs. no HTN.[187] The third trial reported an incomplete and unclear analysis of many subgroup analyses for both EPA+DHA versus placebo and ALA versus placebo. No interaction analyses were reported, but near-significant effects of ALA on MACE reduction were seen for those <70 years old (HR 0.83, P=0.08) as opposed to older subjects (HR 1.00, P=0.98)and for women (HR 0.73, P=0.07) as opposed to men (HR 0.96, P=0.06). Nonsignificant effects of ALA were found in all subgroups based on time since MI, baseline fish intake, baseline EPA+DHA intake, and history of diabetes. Nonsignificant effect of EPA+DHA were found in all subgroups analyzed.

Meta-regression of the marine oil trials found no significant interaction between n-3 FA dose (P=0.15), followup time (P=0.17), or between at risk and CVD populations (P=0.89)

Observational Studies

Seven studies evaluated variously defined MACE (or total CVD events), composite outcomes that combined cardiac, coronary, and cerebrovascular events (**Appendix Table A.3, Figure A.3**). Each study used its own combination of diagnoses. The studies included generally healthy adults or, in one instance, “at risk” adults with hypercholesterolemia on low dose statins.[72, 99, 106, 130, 133, 162, 174, 181] Followup durations ranged from 4 to about 20 years.

n-3 FA Intake

Five studies evaluated n-3 FA intake (Danish National Birth Cohort, Health Professional Follow-up Study, Malmo Diet and Cancer, MESA, Physician's Health Study).[72, 99, 130, 133, 162, 174]

Three studies analyzed intake of total n-3 FA combined (plot # 94 & 95). The Physician's Health Study (in healthy men)[130, 133] and the Malmo Diet and Cancer study (in healthy adults)[99] both found no association with MACE at 4 and 14 years of followup. In contrast the Danish National Birth Cohort (in healthy women who were pregnant at the time of enrollment) found significantly *increased* risks of cerebrovascular, ischemic heart disease, or hypertensive disease hospitalization after 12 years on those with higher n-3 FA intake (plot #95).[162] However, no clear intake threshold was found.

The Malmo Diet and Cancer and MESA studies found no association between ALA intake and MACE at 10 and 14 years of followup (plots #80 & 81).[72, 99]

MESA found a significant association between both EPA, DHA, and DPA intake (separately) and ischemic coronary events, cardiac arrest, stroke, and CVD death in healthy adults after 10 years of followup (plots #83, 86, 92).[72] For DHA intake, the association was near significant for the uppermost quartile with a median dose of 0.15 g/d, for DPA 0.02 g/d, and for EPA 0.04 g/d.

Three studies evaluated combined EPA+DHA or EPA+DHA+DPA intake (plots # 89 & 90). The Health Professionals Follow-up Study (evaluating EPA+DHA)[174] and Malmo Diet and Cancer study (evaluating EPA+DHA+DPA)[99] found no significant association at 14 and 18 years of followup. MESA found a just-statistically-significant lower risk of ischemic coronary events, cardiac arrest, stroke, and CVD death in healthy adults after 10 years of followup with higher intake of EPA+DHA+DPA.[72] The association was near significant for the highest quartile with a median intake dose of about 0.3 g/d.

n-3 FA Biomarkers

Four studies evaluated n-3 FA biomarkers (JELIS, Physician's Health Study, Scottish Heart Health Extended Cohort Study, MESA).[72, 106, 130, 133, 181]

The Physician's Health Study and MESA found no associations between erythrocyte or phospholipid ALA levels and MACE (plot # 82, erythrocyte n-3 FA associations not plotted because they were not analyzed by quantile).[72, 130, 133]

Three studies evaluated EPA biomarkers, two of which found statistically significant associations with MACE (plots #88 & 93). The Physician's Health Study found no significant association between erythrocyte EPA and MACE in healthy men.[130, 133] MESA, in contrast, found a significant association between higher phospholipid EPA and lower MACE (plot #92).[72] In a population of people with dyslipidemia on low-dose statins, JELIS also found a significant association between higher plasma EPA and lower risk of MACE (plot #88).[106]

Four studies evaluated DHA biomarkers, with heterogeneous findings (plots #84 and 85; other biomarkers not plotted due to insufficient reported data or not quantile analysis). JELIS and the Physician's Health Study found no significant associations with plasma or erythrocyte DHA.[106, 130, 133] The Scottish Heart Health Extended Cohort Study, though, found that higher adipose tissue DHA levels were associated with reduced risk of MACE at about 20 years of followup,[181] and the MESA study also found reduced risk of MACE associated with higher phospholipid DHA levels at 10 years of followup.[72]

Three studies evaluated DPA biomarkers, one of which found a significant association (plot #87; other biomarkers not plotted due to insufficient reported data or not quantile analysis). The Scottish Heart Health Extended Cohort Study found that higher adipose tissue DPA levels were associated with lower risk of MACE at about 20 years of followup.[181] In contrast, the

Physician's Health Study and MESA found no significant associations with erythrocyte or phospholipid DPA.[72, 130, 133]

The Physician's Health Study also found no significant association between erythrocyte SDA and MACE.[130, 133]

Two studies evaluated combined EPA+DHA biomarkers (plot #91). The Physician's Health Study found no association with erythrocyte EPA+DHA,[130, 133] but MESA found that higher phospholipid EPA+DHA levels were associated with lower risk of MACE at 10 years of followup.[72]

Observational study subgroup analyses

Only MESA reported subgroup analyses.[72] In comparisons of n-3 FA biomarker associations with MACE by race, the study found no significant differences in associations for EPA, DHA, and EPA+DHA+DPA levels, but whites (HR=0.41) and Chinese (HR=0.30) had significantly stronger associations than African Americans (HR=1.51) and Hispanics (HR=1.33; P interaction = 0.01).

Table A.1. Major Adverse Cardiovascular Events (Composite Outcome): RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs Placebo											
Yokoyama 2007 17398308* Japan	At risk (dyslipidemia; 19.5% with CAD)	EPA+Statin	1.8 g/d (Marine oil)	Statin	0	5 y	Local physicians monitored but compliance level was not reported	262/9326, 2.8%	324/9319, 3.5%	HR 0.81 (0.69, 0.95)	0.01
Einvik 2010 20389249† Scandinavia	At risk	EPA+DHA+diet intervention	2.02 g/d (Marine oil) [E:D 1.4]	Placebo+diet intervention	0 (Corn oil)	3 y	>90% of the tablets were taken based on pharmacy records, and verified by biomarkers	32/282, 11%	36/281, 13%	HR 0.89 (0.55, 1.44)	0.624
Roncaglioni 2013 23656645‡ Italy	At risk	EPA+DHA	0.85 g/d (Marine oil) [E:D 0.9-1.5]	Placebo	0 (Olive oil)	5 y	Monitored by self-report but compliance level was not reported	733/6239, 12%	745/6266, 12%	HR 0.98 (0.88, 1.08)	0.64
Bosch 2012 22686415§ Canada	CVD (or diabetes)	EPA+DHA	0.84 g/d (Marine oil) [E:D 1.24]	Placebo	0 (Olive oil)	6+ y	Followup (adherence was 88% at the end of study)	1034/6281, 16.5%	1017/6255, 5.1%	HR 1.01 (0.93, 1.10)	0.81
Galan 2010 21115589¶ France	CVD	EPA+DHA	0.6 g/d (Marine oil) [E:D 2]	Placebo	0 (nd)	4.7 y	Patient reported (86% reported they took ≥80% of allocated treatment)	81/1253, 7%	76/1248, 6%	HR 1.08 (0.79, 1.47)	0.64
Macchia 2013# 23265344	CVD	EPA+DHA	0.850-0.882 g/d (Marine oil) [E:D 0.5]	Placebo	0 (Olive oil)	1 y	nd	16/289, 6%	20/297, 7%	HR 0.88 (0.44, 1.66)	

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Tavazzi 2008 18757090** Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine oil) [E:D 0.83]	Placebo	0 (nd)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	1981/3494, 57%	2053/3481, 57%	HR 0.92 (0.85, 0.999)	0.009
Kromhout 2010 20929341†† Netherlands	CVD	EPA+DHA (±ALA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±ALA)	0; 2 g/d ALA (Placebo margarine = oleic acid; Plant oil)	3.4 y	90% of the patients adhered fully to the protocol; verified by biomarkers	336/2424, 14.0%	335/2433, 13.8%	HR 0.92 (0.75, 1.13)	0.93
ALA vs. Placebo											
Kromhout 2010 20929341†† Netherlands	CVD	ALA (±EPA+DHA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±EPA+DHA)	0; 0.4 g/d EPA-DHA (placebo = oleic acid; Marine oil) [E:D 3:2]	3.4 y	90% of the patients adhered fully to the protocol; verified by biomarkers	319/2409, 13.2%	352/242,14.5%	HR 0.92 (0.73, 1.11)	0.20

* Sudden cardiac death, fatal and nonfatal myocardial infarction, and other nonfatal events including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting
† Fatal or nonfatal sudden cardiac arrest, myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, cerebral stroke, surgery on abdominal aortic aneurysm, or peripheral revascularization procedures
‡ Death from cardiovascular causes or hospital admission from cardiovascular causes
§ Myocardial infarction, stroke, or death from cardiovascular causes
¶ Nonfatal myocardial infarction, ischemic stroke, or death from cardiovascular disease (including fatal myocardial infarction, stroke, sudden death, aortic dissection, cardiac failure, or other fatal event defined by the medical committee as having a cardiovascular cause)
# First occurrence of either all-cause death, nonfatal stroke, nonfatal acute MI, systemic embolism, heart failure development, or severe bleeding
** Death from any cause or admission to the hospital for cardiovascular reasons
†† Fatal CVD, nonfatal MI, nonfatal cardiac arrest, and nonfatal stroke

Table A.2. Major Adverse Cardiovascular Events (Composite Outcome): Subgroup Analyses, Randomized trials

Study	Population	Subgroups	n-3 FA	Comparator	N Total	P difference	Difference	Favors
Roncaglioni 2013 23656645 Italy	At risk	Men vs. women	EPA+DHA	Placebo	12505	0.04	HR 1.04 vs. 0.82	Women
Yokoyama 2007 17398308 Japan	At risk	Men vs. women	EPA	Placebo	9326	0.43	HR 0.76 vs. 0.87	
		Age ≥61 vs. <61 y	EPA	Placebo	9326	0.57	HR 0.84 vs. 0.76	
		BMI ≥24 vs. <24 kg/m^2	EPA	Placebo	9326	0.88	HR 0.82 vs. 0.80	
		Tg ≥270 vs. <270 mg/dL	EPA	Placebo	9326	0.46	HR 0.76 vs. 0.86	
		Tg ≥150 vs. <150 mg/dL	EPA	Placebo	9326	0.75	HR 0.84 vs. 0.79	
		HDL-c ≥58 vs. <58 mg/dL	EPA	Placebo	9326	0.26	HR 0.96 vs. 0.78	
		LDL-c ≥181 vs. <181 mg/dL	EPA	Placebo	9326	0.83	HR 0.86 vs. 0.82	
		CAD vs. no CAD	EPA	Placebo	9326	0.95	HR 0.81 vs. 0.82	
		Smoker vs. nonsmoker	EPA	Placebo	9326	0.89	HR 0.78 vs. 0.80	
		Diabetes vs. no diabetes	EPA	Placebo	9326	0.62	HR 0.86 vs. 0.79	
		HTN vs no HTN	EPA	Placebo	9326	0.57	HR 0.77 vs. 0.85	
Kromhout 2010 20929341 Scandinavia	CVD	≥70 vs. <70 y	EPA	Placebo	4837	nd	HR 0.97 vs. 1.04	NS both subgroups
		Men vs. women	EPA	Placebo	4837	nd	HR 1.06 vs. 0.82	NS both subgroups
		Time since MI ≥3.7 vs. <3.7 y	EPA	Placebo	4837	nd	HR 1.10 vs. 0.92	NS both subgroups
		Baseline fish intake ≥5 vs. <5 g/d	EPA	Placebo	4837	nd	HR 0.98 vs 1.22	NS both subgroups

Study	Population	Subgroups	n-3 FA	Comparator	N Total	P difference	Difference	Favors
		Baseline EPA+DHA intake ≥50 vs. <50 mg/d	EPA	Placebo	4837	nd	HR 0.99 vs 1.15	NS both subgroups
		Diabetes vs. no diabetes	EPA	Placebo	4837	nd	HR 0.78 vs 1.10	NS both subgroups
Kromhout 2010 20929341 Scandinavia	CVD	≥70 vs. <70 y	ALA	Placebo	4837	nd	HR 1.00 vs. 0.83	NS older P=0.08 younger
		Men vs. women	ALA	Placebo	4837	nd	HR 0.96 vs. 0.73	NS men P=0.07 women
		Time since MI ≥3.7 vs. <3.7 y	ALA	Placebo	4837	nd	HR 0.91 vs. 0.92	NS both subgroups
		Baseline fish intake ≥5 vs. <5 g/d	ALA	Placebo	4837	nd	HR 0.93 vs. 0.84	NS both subgroups
		Baseline EPA+DHA intake ≥50 vs. <50 mg/d	ALA	Placebo	4837	nd	HR 0.91 vs. 0.94	NS both subgroups
		Diabetes vs. no diabetes	ALA	Placebo	4837	nd	HR 0.91 vs. 0.91	NS both subgroups

Figure A.2. Major adverse cardiovascular events: Randomized trials of marine oils

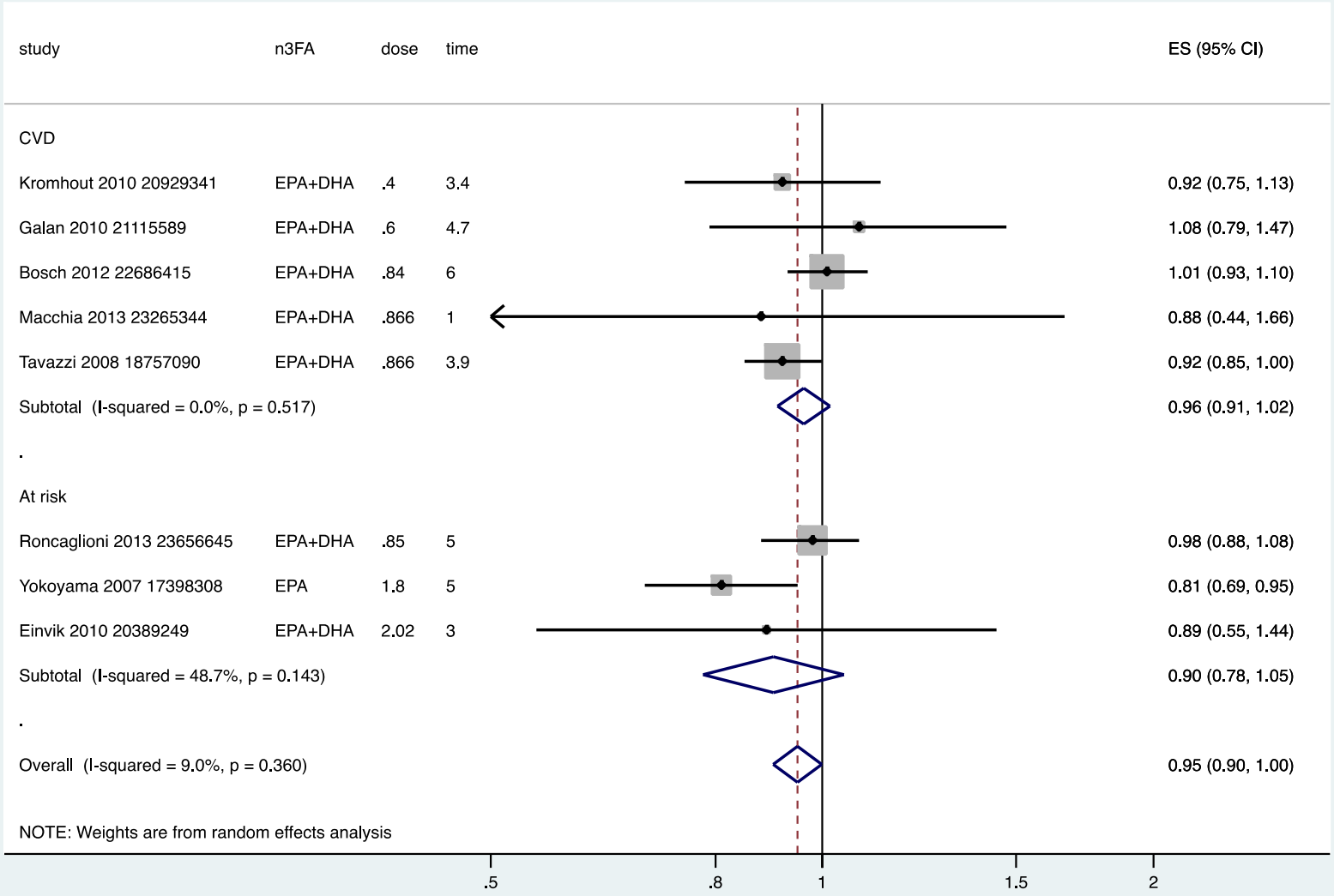
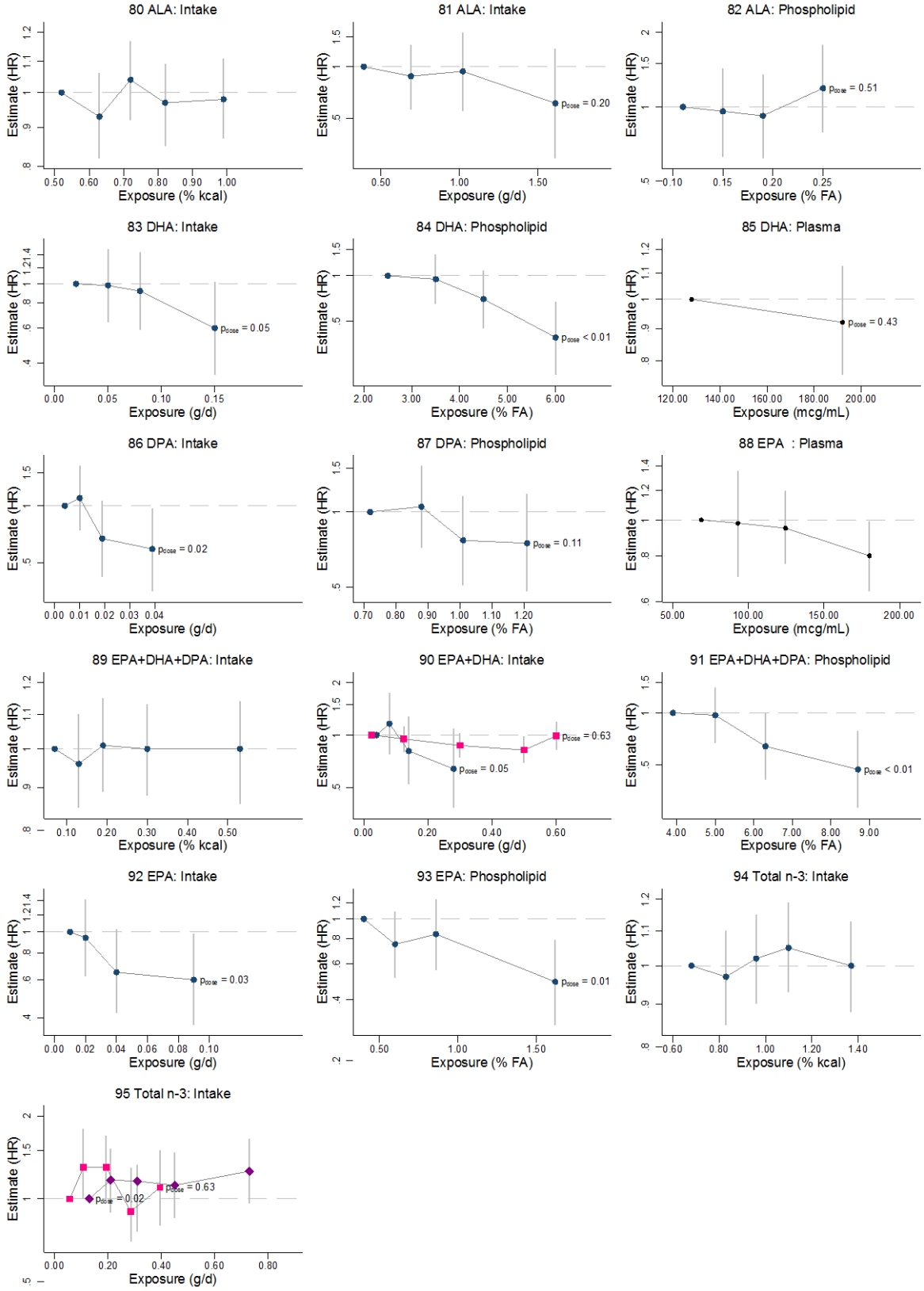


Figure A.3. n-3 FA associations with major adverse cardiovascular events: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles. Where 95% confidence intervals (vertical lines) are missing, these were not reported in the studies.
Blue circles = healthy adults, black circles = adults with dyslipidemia (at risk), pink squares = healthy males, purple diamonds = healthy females.

CVD Death (Including Stroke)

Randomized Controlled Trials

Six RCTs reported total CVD death (**Table B.1**).[56, 78, 114, 126, 153, 168] Of these, two were conducted in a total of 13,068 people at risk of CVD defined as dyslipidemia or at least four CVD risk factors,[78, 153] and the other four in a total of 29,270 people with CVD including DM, history of CVD, MI or heart failure.[56, 114, 126, 168]

Marine oil vs. placebo

Meta-analysis of the six RCTs of marine oil versus placebo yielded a near-significant summary effect size for risk of CVD death: HR=0.91 (95% CI 0.81, 1.01; P=0.073) (**Figure A.2**).[56, 78, 114, 126, 153, 168]

At risk for CVD population

Among people at risk of CVD, two studies compared marine oil (EPA+DHA) to placebo (either olive oil or corn oil) in a total of 13,068 participants.[78, 153] The doses of EPA and DHA were less than 0.85 and 2.02 g/d, and the EPA to DHA ratio ranged from 0.9 to 1.5. Compliance was high (>90%) in one study[78] and not reported (although monitored by self-report) in another study.[153] The durations of followup were 3 and 5 years. Both studies found that EPA+DHA supplementation did not significantly reduce CVD death compared with placebo (HR 1.03, 95% 0.82, 1.30; OR 0.62, 95% CI 0.24, 1.64).

Subgroup meta-analysis yielded a summary HR of 0.95 (95% CI 0.65, 1.37).

CVD population

Among people with CVD, four trials compared marine oil (EPA+DHA) to placebo (olive oil in one study and source was not reported in another study),[56, 168] , to no intervention[126] and in a factorial study with ALA,[114] in a total of 24,433 participants. The dose of EPA+DHA ranged from 0.84 to 0.88 g/d, and the EPA to DHA ratio ranged from 0.5 to 1.24. Compliance ranged from about 70 to 88 percent. The mean duration of followup ranged from 3.5 to more than 6 years. Two of the three studies found that EPA+DHA supplementation significantly reduced the CVD death compared with no intervention or placebo in 11,334 participants with MI (RR 0.70, 95% CI 0.56, 0.86)[126] and in 6975 participants with heart failure (adjusted HR 0.91, 95% CI 0.81, 0.99).[168] The third study did not find a difference in the risk of CVD death between EPA+DHA and placebo in 12,536 participants with DM or history of CVD (HR 0.98, 95% CI 0.87, 1.10).[56] The fourth study was the 2-by-2 factorial RCT described under *Major Adverse Cardiovascular Events* that compared EPA+DHA, EPA+DHA and ALA, ALA, and oleic acid margarines in 4837 participants with MI.[114] . During a mean of 3.4 years of followup, EPA+DHA containing margarines had no significant effect on CVD death compared with the ALA alone or placebo margarines (HR 0.98; 95% CI 0.72, 1.33).

Subgroup meta-analysis yielded a summary HR of 0.89 (95% CI 0.78, 1.01).

ALA vs. placebo

CVD population

In the 2-by-2 factorial RCT, the groups that received ALA margarines had no significant difference in the risk of MACE compared with placebo margarines (HR 0.94; 95% CI 0.69, 1.27).[114]

RCT subgroup analyses

The same 2-by-2 factorial RCT analyzed subgroups based on history of diabetes.[114] For patients with diabetes, EPA+DHA had a near significant effect on CVD death (HR=0.60, P=0.08) in contrast to those without diabetes (HR=1.21, P=0.32); no test for interaction was reported. The effect of ALA on CVD death was similarly nonsignificant in both patients with diabetes (HR=0.87, P=0.63) and those without diabetes (HR=0.97, P=0.87).

Meta-regression of the marine oil trials found no significant interaction between n-3 FA dose (P=0.34), followup time (P=0.30), or between at risk and CVD populations (P=0.51)

Observational Studies

Eight studies evaluated the association between n-3 FA intake or biomarkers and total CVD death in healthy adults from 4 to 31 years of followup (median 11 years) (**Appendix Table B.3, Figure B.4**).[75, 85, 132-134, 136, 164, 179, 185] The studies had heterogeneous findings regarding associations between higher n-3 FA intake or biomarker levels and lower risk of CVD death.

n-3 FA Intake

Six studies evaluated n-3 FA intake (JACC, MRFIT, NIPPON DATA80, Physician's Health Study, Shanghai Women's and Men's Health Studies, Takayama).[75, 130, 132, 133, 136, 164, 185]

Three studies evaluated total n-3 FA intake (JACC, NIPPON DATA80, Physician's Health Study) (plots #136 & 137).[130, 132, 133, 185] JACC found a significant association between higher total n-3 FA intake (combined) and lower CVD death risk in healthy adults after about 13 years of followup, with a significant association occurring in quantile with median of 2 g/d or higher.[185] JACC and NIPPON DATA80, however, found no significant associations at 4 and 24 years of followup.[132, 185]

Two studies evaluated ALA intake with conflicting results (plots #124 & 125). MRFIT found a significant association between higher ALA intake (measured as percent Kcal) and lower CVD risk at about 10 years (particularly in quartiles with median intake greater than about 0.7% Kcal), but a nonsignificant association (P<0.10) when ALA intake was measured as g/day. The Cardiovascular Health Study found no association at 12 years of followup.[75]

Two studies evaluated EPA intake, also with conflicting results (plots #133 & 134). NIPPON DATA80 found no association at 24 years of followup,[132] but the Shanghai Women's and Men's Health Studies found a significant association between higher EPA intake and lower risk of CVD death among men (at about 6 years of followup) and women (at about 12 years), combined (with significant associations in all quintiles with median intake of about 0.01 g/d or higher).[164]

The same two studies evaluated DHA intake (plots #127 & 128). NIPPON DATA found a near significant association between higher DHA intake and lower CVD death risk

(P=0.099).[132] The Shanghai Women's and Men's Health Studies found a significant association between higher EPA intake and lower risk of CVD death, as with EPA.[164] Significant or near-significant associations were seen in quantiles with median doses of about 1.25 percent Kcal or about 0.02 g/d, or higher.

Four studies evaluated EPA+DHA (3 studies; NIPPON DATA80, Shanghai Women's and Men's Health Studies, Takayama)[132, 136, 164] or EPA+DHA+DPA (MRFIT) (plots #131 & 132).[75] The Shanghai Women's and Men's Health Studies and MRFIT found significant associations between higher marine oil intake and lower CVD death risk.[75, 164] In MRFIT, the association was statistically significant when marine oil intake (either g/day or % Kcal) was analyzed as a continuous variable in a linear model and near-significant (P<0.10) when analyzed across quintiles.[75] Both NIPPON DATA80 and Takayama found no significant associations.[132, 136] For percent Kcal analyses, near significant associations were found in quantiles with median intake of about 0.30 percent Kcal or higher. In two of the g/d analyses, near-significant associations were found in quantiles with median marine oil intake of about 0.7 g/d.

n-3 FA Biomarkers

The Cardiovascular Health Study and ULSAM evaluated n-3 FA plasma levels.[117, 179]

The Cardiovascular Health Study found a significant association between higher total n-3 FA plasma levels and lower risk of CVD death (plot #138).[117]

Both the Cardiovascular Health Study and ULSAM found no association between plasma ALA levels and CVD death risk (plot # 126).[117, 179]

For both plasma EPA and DHA levels (separately), the Cardiovascular Health Study found significant associations between higher plasma levels and lower risk of CVD death at 16 years of followup.[117] In contrast, ULSAM found no significant association at about 31 years of followup (plots #129 & 135).[179]

The Cardiovascular Health Study also found a significant association between higher plasma DPA levels and lower risk of CVD death (plot #130).

Observational study subgroup analyses

Only the Cardiovascular Health Study reported subgroup analyses.[117] In their analysis of ALA intake, they reported no significant difference (without details) in association between participants with high, low, or no fish consumption and between men and women.

Table B.1. CVD Death (Including Stroke): RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo											
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA (±ALA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±ALA)	0; 2 g/d ALA (Placebo margarine = oleic acid; Plant oil)	3.4 y	90% of the patients adhered fully to the protocol; verified by biomarkers	80/2424, 3.3%	82/2433, 3.4%	HR 0.98 (0.72, 1.33)	0.89
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	≤0.85 g (Marine oil) [E:D 0.9-1.5]	Placebo	0 (Olive oil)	5 y	Monitored by self-report but compliance level was not reported	142/6239, 2.3%	137/6266, 2.2%	HR 1.03 (0.82, 1.30)	0.8
Einvik 2010 20389249 Norway	At risk	EPA+DHA+ diet intervention	2.02 g/d (Marine oil) [E:D 1.4]	Placebo+diet intervention	0 (Corn oil))	3 y	>90% of the tablets were taken based on pharmacy records, and verified by biomarkers	7/282, 2%	11/281, 4%	OR 0.62 (0.24, 1.64)[c]	nd
Bosch 2012 22686415 Canada	CVD[d]	EPA+DHA	0.84 g/d (Marine oil) [E:D 1.24]	Placebo	0 (Olive oil)	6+ y	Followup (adherence was 88% at the end of study)	574/6281, 9.1%	581/6255, 9.3%	HR 0.98 (0.87,1.10)	0.72
Marchioli 2002 11997274 Italy	CVD	EPA+DHA	0.850- 0.882 g/d (Marine oil) [E:D 0.5]	No intervention	nd	3.5 y	Followup (adherence was 72.5% at the end of study)	310/5666, 5.5%	370/5668, 6.5%	RR 0.70 (0.56, 0.86)	<0.001

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine oil) [E:D 0.83]	Placebo	0 (nd)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	712/3494, 20.4%	765/3481, 22.0%	Adjusted HR 0.90 (0.81, 0.99)[b]	0.045
ALA vs. Placebo											
Kromhout 2010 20929341 Netherlands	CVD	ALA (±EPA+DHA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±EPA+DHA)	0; 0.4 g/d EPA-DHA (placebo = oleic acid; Marine oil) [E:D 3:2]	3.4 y	90% of the patients adhered fully to the protocol; verified by biomarkers	78/2409, 3.2%	84/2428, 3.5%	HR 0.94 (0.69, 1.27)	0.67

Figure B.2. CVD death: Randomized trials of marine oils

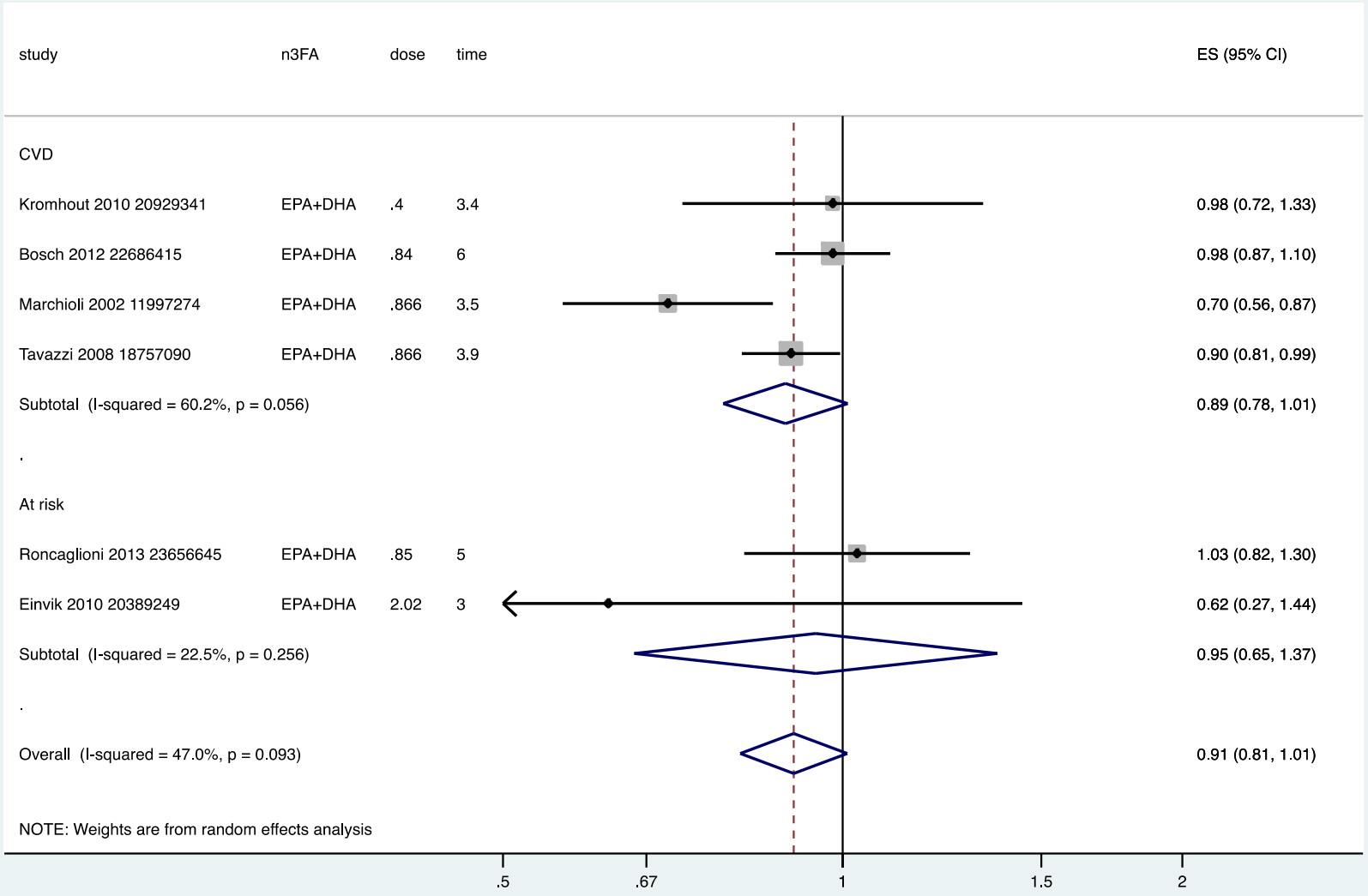
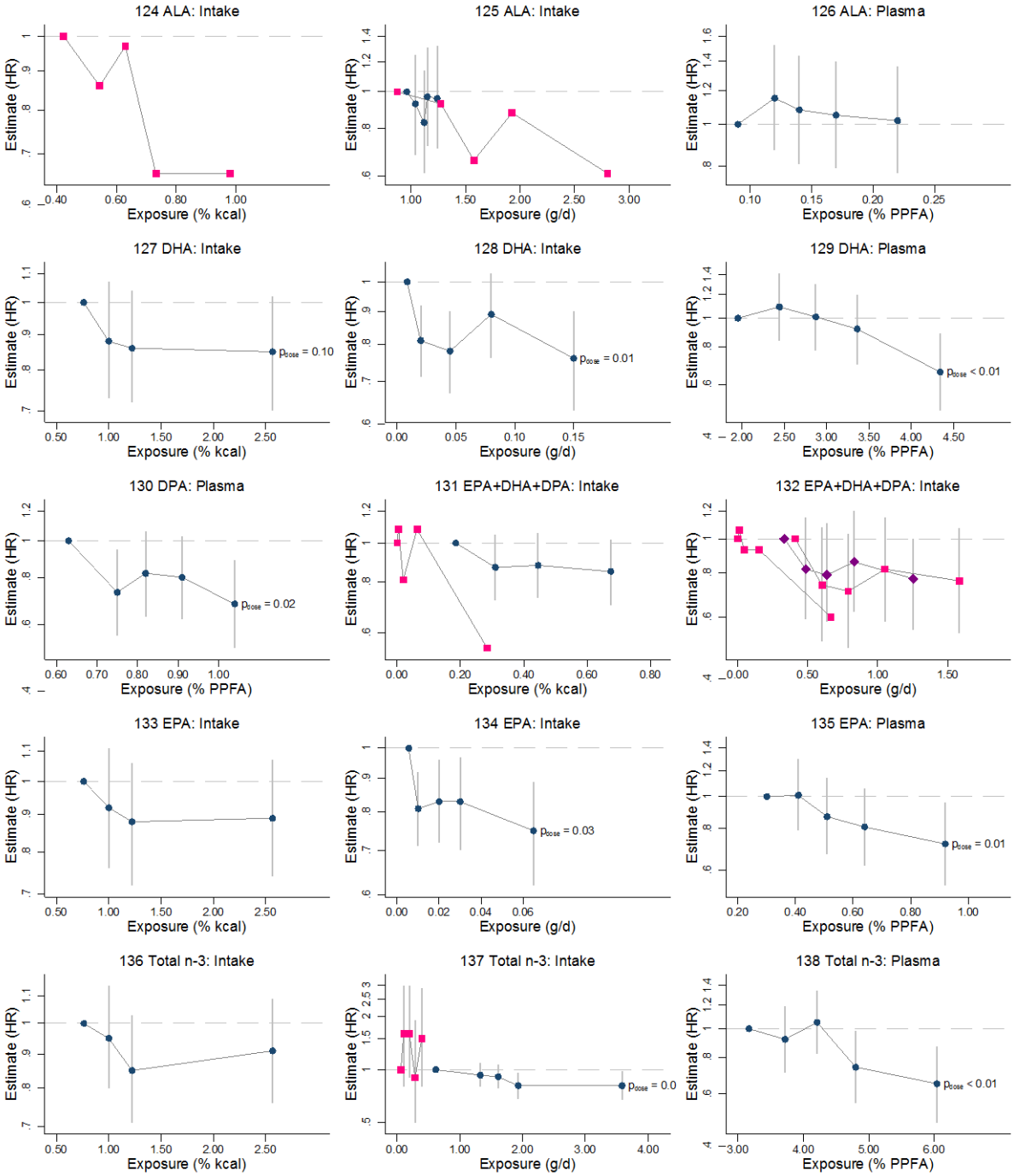


Figure B.3. n-3 FA associations with CVD death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles. Where 95% confidence intervals (vertical lines) are missing, these were not reported in the studies.
Blue circles = healthy adults, pink squares = healthy males, purple diamonds = healthy females.

Cardiac Death

Randomized Controlled Trials

Four RCTs reported on cardiac death (combined coronary heart disease [CHD] and other cardiac death) (**Table C.1**).[59, 61, 116, 126] The trials were conducted in a total of 15,596 people with CVD including MI, arrhythmia, CAD.

Marine oil vs. placebo

CVD population

Among people with CVD, three compared marine oil (EPA+DHA) to placebo (oleic acid or olive oil) or no intervention in a total of 12,282 participants with arrhythmia, MI or CAD,[59, 116, 126] and one compared two levels of "fish advice" (dietician to advise to increase fish and/or fish oil supplement intake) with no fish advice in a total of 3114 men with MI or angina.[61]

Among the three RCTs that compared marine oil (EPA+DHA) to placebo (oleic acid or olive oil) or no intervention EPA+DHA ranged from 0.8 to 2.6 g/d. In the one RCT reporting sufficient details, the EPA to DHA ratio was 1.4. Compliance was generally good (>70%). The duration of follow-up ranged from 1 to 3.5 years. Two of the three RCTs found that EPA+DHA supplementation did not have significant effects on cardiac death (OR=0.45 and 1.01).[59, 116] The third RCT found that EPA+DHA supplementation had protective effects against cardiac death (RR 0.65; 95% CI 0.51, 0.82).[126]

In the study that compared "fish advice" (advise to increase fish intake in one subgroup and additional advise to take fish oil supplement in a second subgroup) with "no fish advice",[61] the mean EPA intake estimated by the dietary assessment was 0.45 and ≤0.85 g/d in the "fish advice" groups, and was 0.11 in the "no fish advice" group. No estimates for DHA intake levels were reported. Compliance was good (fish intake was significantly increased in the "fish advice" groups) based on the dietary assessments. The trial found that, after 9 years of followup, overall, there was a significant *increase* in cardiac death between 1571 men with angina who were advised to increase fish intake and 1543 men with angina who were not (adjusted HR 1.26; 95% CI 1.00, 1.58; P=0.047). The effect was similar but nonsignificant in the subgroup of 1109 men given advice only about increasing fish intake (adjusted HR 1.20, 95% CI 0.93, 1.53) but larger and statistically significant in 462 men who were advised to take a fish oil supplement (adjusted HR 1.45; 95% CI 1.05, 1.99).[61]

RCT subgroup analyses

The RCT that found a significant increased risk of cardiac death with combined fish diet and EPA+DHA supplements reported subgroup analyses for cardiac death.[61] It found nonsignificant interactions between fish advice and the following five pairs of subgroups, based on whether they take nitrates, digoxin, lipid-lowering drugs, anticoagulants, or diuretics.

Observational Studies

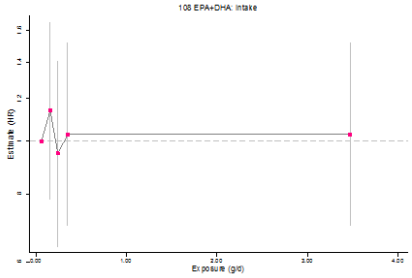
Two studies evaluated a composite outcome of fatal coronary heart disease and sudden death, both in healthy adult males (**Appendix Table C.3, Figure C.4**).[49, 130] The Health Professionals Follow-up Study found no association between EPA+DHA intake and cardiac

death (plot #108). The Physician's Health Study found no associations between erythrocyte ALA, EPA, DHA, DPA, SDA, or EPA+DHA+DPA levels and cardiac death.[130, 133]

Table C.1. Cardiac death: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo											
Brouwer 2006 16772624 N.Europe	CVD	EPA+DHA	0.96 g n-3 PUFAs (0.464 g EPA, 0.335g DHA) (Marine oil) [E:D]=1.4	Placebo	0 (high-oleic acid sunflower oil)	1 y	Generally good (76% reported taking 80% pills) based on pill counts and confirmed by biomarkers.	6/273, 2%	13/273, 5%	OR 0.45 (0.17, 1.20)	0.111
Leaf 2005 16267249 US	CVD	EPA+DHA	EPA plus DHA of 2.6 g (Marine oil)	Placebo	0 (Olive oil)	12 mo	Pill counts and analysis of the phospholipids of red blood cells for their content of EPA and DHA	9/200, 4.5%	9/202, 4.5%	OR 1.01 (0.39, 2.60)	0.983
Marchioli 2002 11997274 Italy	CVD	EPA+DHA	EPA and DHA 0.850- 0.882 g/d) (Marine oil)	No intervention	nd	3.5 y	Followup (adherence was 72.5% at the end of study)	247/5666, 4.4%	306/5668, 5.4%	RR 0.65 (0.51, 0.82)	<0.001
Burr 2003 12571649 UK	CVD	Fish advice	EPA 0.45 g/d (diet)[a]	No fish advice	EPA 0.11 (diet)[a]	9 y	dietary charts sent by post with reply-paid envelopes	121/1109, 10.9%	139/1543, 9.0%	Adj HR 1.20 (0.93, 1.53)	0.16
		EPA+DHA (advice to take fish oil)	EPA ≤0.51 and DHA ≤0.345 (marine oil)[a]	No fish advice	EPA 0.11 (diet)[a]	9 y	dietary charts sent by post with reply-paid envelopes	85/462, 18.4%	139/1543, 9.0%	Adj HR 1.45 (1.05, 1.99)	0.024

Figure C.4. n-3 FA associations with cardiac death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for cardiac death. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted.
Pink squares = healthy males.

Coronary Heart Disease Death

Randomized Controlled Trials

Four RCTs evaluated CHD (or coronary artery disease) death (**Table D.1**).[62, 114, 155, 187] Of these, one study was conducted in 18,645 participants with dyslipidemia (19.5% with CAD),[187] and three were conducted in a total of 6929 people with CVD including MI, arrhythmia, CAD.

Marine oil vs. placebo

At risk for CVD population

Among people at risk of CVD, one study compared 1.8 g/d EPA ethyl ester combined with statin with control (statin alone) in 18,645 participants with dyslipidemia (19.5% with CHD).[187] Local physicians monitored compliance with dietary advice and medication at every clinic visit but the adherence level was not reported. This study found no significant additive effect of EPA supplementation on risk of CHD death compared with statin alone (HR 0.94; 95% CI 0.57, 1.56).

CVD population

Among people with CVD, two studies compared marine oil (EPA+DHA) to placebo (oleic acid or olive oil) in a total of 4896 participants with arrhythmia, MI or CAD,[114, 155] one was the 2-by-2 factorial RCT described under *Major Adverse Cardiovascular Events*.[114]

A relatively small trial (with 59 participants) compared 6 g/d marine oil (2.88 g/d EPA, 1.92 g/d DHA, 1.2 g/d DPA) to olive oil placebo for 2.4 years, with 80 percent compliance in the marine oil supplement arm (and 90% compliance in the olive oil placebo arm).[155] The 2-by-2 factorial trial compared 0.4 g/d of EPA+DHA in margarine to placebo margarine for 40 months with 90 percent compliance, overall.[114] Both trials found no significant association between marine oil intake and CHD death, but the smaller trial had only one such death during its followup.

In one trial that compared “fish advice” (advise to increase fish intake) with “no fish advice” in 2033 adults,[62] the mean EPA intake estimated by the dietary assessment was 0.34 g/d in the “fish advice” group and 0.09 in the “no fish advice” group. No estimates for DHA intake levels were reported. Compliance was good based on the dietary assessments. No significant difference in risk of CHD death was found (adjusted HR = 0.92; 95% CI 0.66, 1.29).

ALA vs. placebo

CVD population

The 2-by-2 factorial study compared 2 g/d ALA in margarine to control margarine.[114] The trial found no difference in risk of CHD death after 40 months (HR 0.92; 95% CI 0.66, 1.29).

RCT subgroup analyses

The 2-by-2 factorial study found significant protective effect of EPA+DHA in subjects with diabetes (HR=0.51, P=0.04) that was not seen in subjects without diabetes (HR=1.21, P=0.32); no analysis of a statistical interaction was reported.[114] In both subgroups, the effect of ALA on CHD death was nonsignificant (HR=0.87, P=0.63 with diabetes; HR=0.97, P=0.87 without diabetes.

In the trial of participants with dyslipidemia (19.5% of whom had CHD),[187] no significant effect of EPA was found. In participants with no history of CHD (primary prevention), HR=1.00 (95% CI 0.32, 3.11). In participants with a history of CHD (secondary prevention), HR=0.64 (95% CI 0.21, 1.94).

Observational Studies

Ten studies evaluated associations between n-3 FA intake and biomarkers and CHD death, including the Pooling Project, which pooled data from eight large cohorts (ARIC, FMC, IWHS, NHS, VIP, WHS, ATBC, HPFS) (**Appendix Table D.3, Figure D.4**).[68, 75, 85, 102, 104, 132, 134, 147, 164, 173, 185] The studies were all conducted in healthy adults with average followup ranging from about 6 to 24 years (median 11.3 years).

n-3 FA Intake

All 10 studies analyzed n-3 FA intake (Alpha-Tocopherol Beta-Carotene Cancer Prevention, Cardiovascular Health Study, JACC, Japan Public Health Center-Based Study - Cohort I, MORGEN, MRFIT, NIPPON DATA80, Nurses' Health Study, Pooling Project of Cohort Studies on Diet and Coronary Disease, Shanghai Women's and Men's Health Studies).

The NIPPON DATA80 and JACC studies found no associations between total n-3 FA intake (combined) and CHD death after 13 and 24 years of followup (plots #121 & 122).[132, 185]

Four studies, including the Pooling Project and thus comprising eight study cohorts, evaluated ALA intake (Alpha-Tocopherol Beta-Carotene Cancer Prevention, Cardiovascular Health Study, MRFIT, Pooling Project of Cohort Studies on Diet and Coronary Disease) (plots #109 & 110).[75, 134, 147, 173] MRFIT found a statistically significant association between higher ALA intake measured as percent Kcal (energy) in men after about 10 years of followup (with possibly significant associations bound in quartiles with median values above about 0.5% Kcal), but no association with ALA intake measured as g/day.[75] The other three studies also found no association (in men, women, or all healthy adults) at 6, 12, and 4-10 years of followup.

Two studies (NIPPON DATA80, Shanghai Women's and Men's Health Studies) found no associations with EPA or DHA intake (separately) and CHD death at 24 years in one study and at about 6 years in men and 11 years in women in the other study (plots #112, 113, 118 & 119).[132, 164]

Seven studies analyzed EPA+DHA (5 studies; Japan Public Health Center-Based Study - Cohort I, NIPPON DATA80, Nurses' Health Study, MORGEN, Shanghai Women's and Men's

Health Studies[70, 102, 104, 132, 164]) or EPA+DHA+DPA (2 studies; Alpha-Tocopherol Beta-Carotene Cancer Prevention, MRFIT [75, 147]) between 6 and 24 years of followup.[75] The studies found heterogeneous results (plots #116 & 117). Three studies (MORGEN, MRFIT, Nurses' Health Study) found significant associations between higher EPA+DHA±DPA and lower risk of CAD death (with significant associations occurring in quantiles with median intake of at least about 0.1% Kcal or 0.25 g/d).[70, 75, 102] One study found a nonsignificant increase in risk of CAD with higher EPA+DHA intake (Japan Public Health Center-Based Study - Cohort I, P=0.10).[104] The remaining three studies found no associations between EPA+DHA±DPA and CAD death risk. Meta-analysis could not be run because intake was inconsistently measured as either g/d or percent Kcal.

n-3 FA Biomarkers

The Cardiovascular Health Study was the only study to evaluate the association between n-3 FA biomarkers and CAD death.[134] At 16 years of followup, higher plasma total n-3 FA and higher plasma DHA were each significantly associated with lower risk of CAD death. No associations were found for ALA, EPA, or DPA plasma levels (plots #111, 114, 115, 120, and 123).

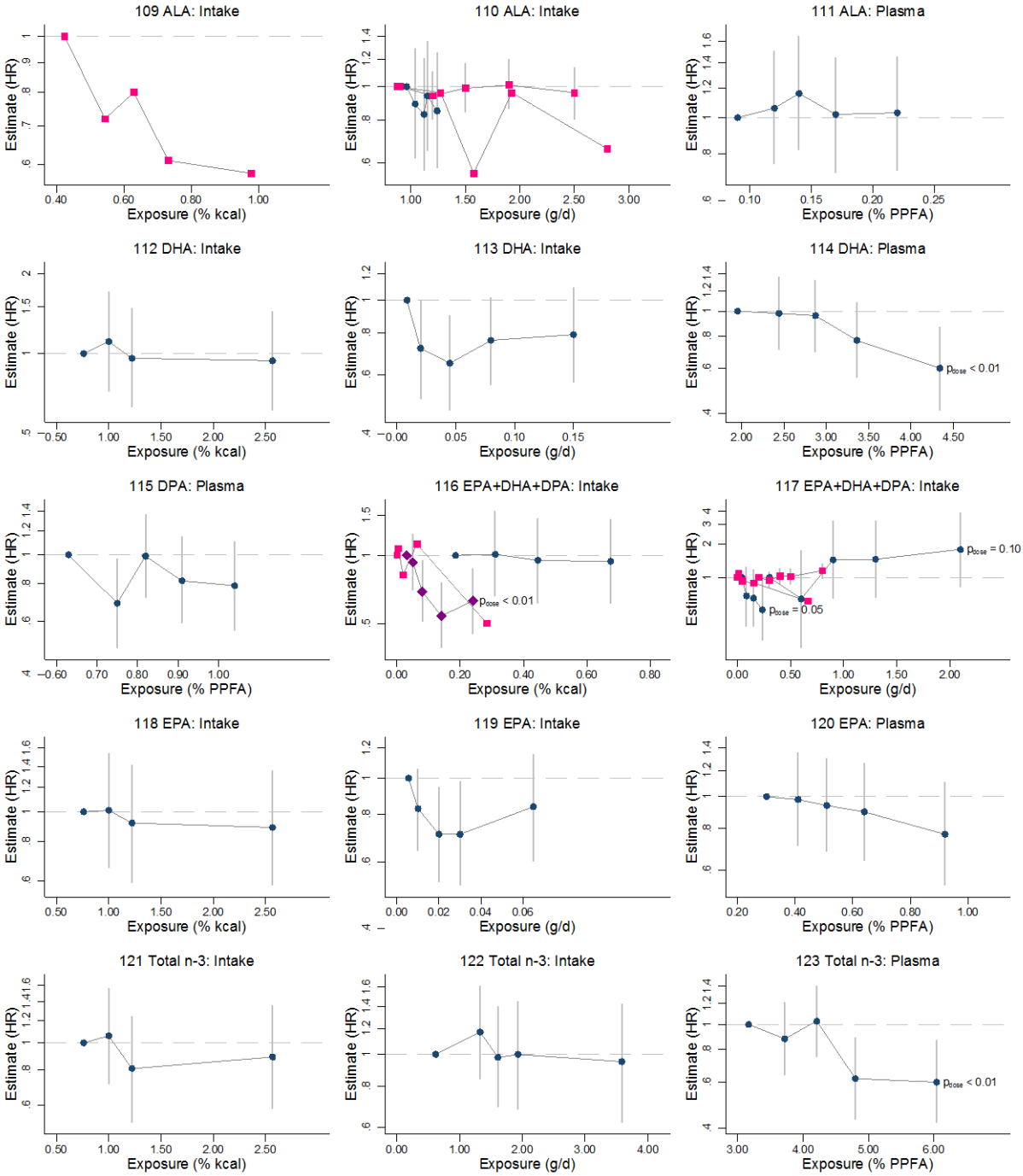
Observational study subgroup analyses

The Pooling Project analysis of ALA, found a near-significant interaction by sex (P=0.07), such that higher ALA intake was protective against CHD death in men (HR=0.77; 95% CI 0.58, 1.01) but not in women (HR=0.88; 95% CI 0.68, 1.14).[173]

Table D.1. CHD death: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo											
Yokoyama 2007 17398308 Japan	At risk (dyslipidemia; 19.5% with CAD)	EPA+Statin	EPA 1.8 g/d (Marine oil)	Statin	0	5 y	Local physicians monitored but compliance level was not reported	29/9326, 0.3%	31/9319, 0.3%	HR 0.94 (0.57, 1.56)	0.812
Sacks 1995 7759696 US	CVD	EPA+DHA+DPA	6 g/d (suppl) [E:D 1.5]	Placebo	0 (Olive oil)	2.4 y	Pill counting (80% for EPA+DHA; 90% for placebo)	0/31, 0.0%	1/28, 3.6%	RD −3.6% (−10.4%, 3%)	0.309
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA (±ALA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±ALA)	0; 2 g/d ALA (Placebo margarine = oleic acid; Plant oil)	40 mo	90% of the patients adhered fully to the protocol; verified by biomarkers	67/2404, 2.8%	71/2433, 2.9%	HR 0.95 (0.68,1.32)	0.75
Burr 1989 2571009 UK	CVD	Fish advice, either alone or in combination with fiber advice, fat advice, or both fiber and fat advice.	EPA 0.34 g/d (diet)	No fish advice (Fat advice, fiber advice, fiber and fat advice, or no advice)	EPA 0.09 g/day (diet)	Overall years (10+ y)	Compliance was good based on dietary assessments	354/1015, 34.9%	384/1018, 37.7%	Adj HR 0.92 (0.80, 1.07)	NS
ALA vs. Placebo											
Kromhout 2010 20929341 Netherlands	CVD	ALA (±EPA+DHA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±EPA+DHA)	0; 0.4 g/d EPA-DHA (placebo = oleic acid; Marine oil) [E:D 3:2]	40 mo	90% of the patients adhered fully to the protocol; verified by biomarkers	66/2409, 2.7%	72/2428, 3.0%	HR 0.92 (0.66, 1.29)	0.64

Figure D.4. n-3 FA associations with CHD death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles. Where 95% confidence intervals (vertical lines) are missing, these were not reported in the studies.
Blue circles = healthy adults, pink squares = healthy males, purple diamonds = healthy females.

Myocardial Infarction Death

Randomized Controlled Trials

No RCTs evaluated this outcome.

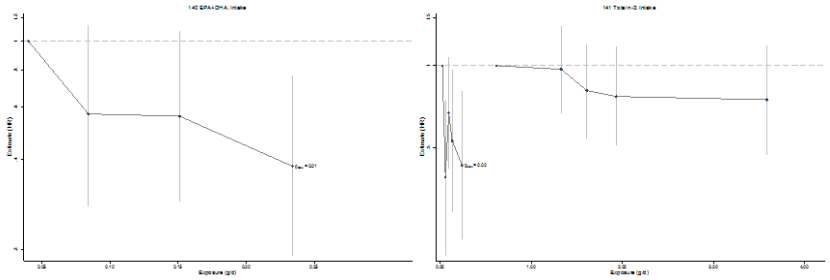
Observational Studies

Three studies evaluated n-3 FA and myocardial infarction (MI) death in healthy adults (**Appendix Table E.3, Figure E.3**).[68, 185, 188] The Shanghai study found a significant association between higher total n-3 FA intake and lower risk of MI death at 12 years of followup, with significant associations found in quintiles with median intake above about 0.05 g/d).[164] In contrast, JACC found no association between total n-3 FA intake and MI death at about 13 years of followup.[185] In a single analysis of EPA+DHA intake, MORGEN found a significant association between higher EPA+DHA intake and lower risk of MI death at about 11 years of followup, with a significant association found in the quartile with intake >0.19 g/d.[70]

Observational study subgroup analyses

The Shanghai study reported no difference in association (with total n-3 FA intake) by baseline total cholesterol to HDL-c ratio.[164]

Figure E.3. n-3 FA associations with myocardial infarction death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for myocardial infarction death.
P values are the study-reported P value for the trend across quantiles.
Blue circles = healthy adults.

Congestive Heart Failure Death

Randomized Controlled Trials

Marine oil vs. placebo

At risk for CVD population

One trial in 12,505 participants at risk for CVD based on multiple risk factors compared a marine oil supplement with at least 0.85 g/d EPA+DHA with olive oil placebo (**Table F.1**).[153] The EPA to DHA ratio ranged from 0.9 to 1.5. Compliance data were not reported. After 5 years of followup, no effect on CHF death was seen (HR=1.00; 95% CI 0.53, 1.88).

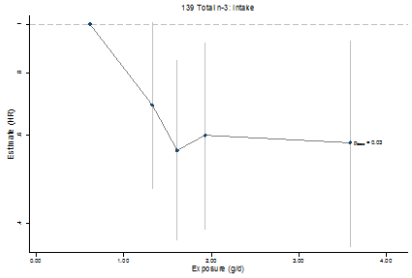
Observational Studies

Only JACC evaluated n-3 FA and CHF death (**Appendix Table F.3, Figure F.4**).[185] In healthy adults, the study found a significant association between higher total n-3 FA intake (combined) and lower risk of CHF death after about 13 years of followup, with significant associations found in quintiles with intake >2.1 g/d.

Table F.1. Congestive Heart Failure Death: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo											
Roncaglioni 2013 23656645 Italy	At risk	EPA+ DHA	≥0.85 g/d (suppl) [E:D 0.9-1.5]	Placebo	0 (Olive oil)	5 y	Self-reported (nd on level of adherence)	19/6239, 0.3%	19/6266, 0.3%	HR 1.00 (0.53, 1.88)	0.99

Figure F.4. n-3 FA associations with heart failure death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for heart failure death. P values are the study-reported P value for the trend across quantiles.
Blue circles = healthy adults.

Stroke Death, Total (Ischemic and Hemorrhagic)

Randomized Controlled Trials

Three RCTs evaluated total stroke death (**Table G.1**).[62, 153, 168] One trial was in 12,505 participants at risk for CVD based on multiple risk factors,[153] and the other two were in a total of 9008 participants with a history of MI,[62] or heart failure.[168]

Marine oil vs. placebo

At risk for CVD population

One RCT evaluated the effect of marine oil (EPA+DHA) on stroke death compared with placebo (olive oil) in a total of 12,505 participants with high risk for CVD.[153] The dose of EPA+DHA was at least 0.85 g/d (composition of the marine oil was not reported). Adherence was verified by participants' self-report but the level of adherence was not reported. After 5 years, the study found no significant difference in stoke death comparing EPA+DHA with placebo (HR 1.05, 95% CI 0.55-2.00).[153]

CVD population

One trial compared marine oil (EPA+DHA) supplementation (0.85-0.88 g/d) to placebo in 6975 participants with heart failure.[168] After 3.9 years of followup, about 30 percent of participants in both study arms were not taking the supplement. No difference was found in risk of stroke death (OR = 1.13; 95% CI 0.75, 1.71). A second trial compared fish advice (resulting in an average of 0.34 g/d EPA intake) with no fish advice (0.09 g/d EPA intake) in 2033 adults with a history of MI.[62] Compliance was not reported. After more than 10 years of followup, no significant difference in stroke death was found (OR=1.23; 95% CI 0.71, 2.14).

Observational Studies

Four studies evaluated n-3 FA intake and biomarkers and risk of total stroke death at 12 to 24 years of followup in healthy adults (**Appendix Table G.3, Figure G.4**).[132, 134, 185, 188]

n-3 FA Intake

Three studies evaluated n-3 FA intake and risk of stroke death (JACC, NIPPON DATA80, Shanghai).[132, 185, 188] All analyses were nonsignificant, including for total n-3 FA (combined) intake (all three studies) at 12, 13, and 24 years of followup (plots #153 & 154); and EPA, DHA, and EPA+DHA intake (separately) in the NIPPON DATA80 study at 24 years of followup (plots #147, 150, & 151).[132]

n-3 FA Biomarkers

Only the Cardiovascular Health Study evaluated n-3 FA biomarkers.[134] The study found near significant associations between higher plasma total n-3 FA (plot #155), DHA (plot #148), and DPA levels (plot #149), separately, and lower risk of stroke death after 16 years of followup (P=0.092, 0.082, and 0.056, respectively). The study found no association with plasma EPA levels (plot #152).

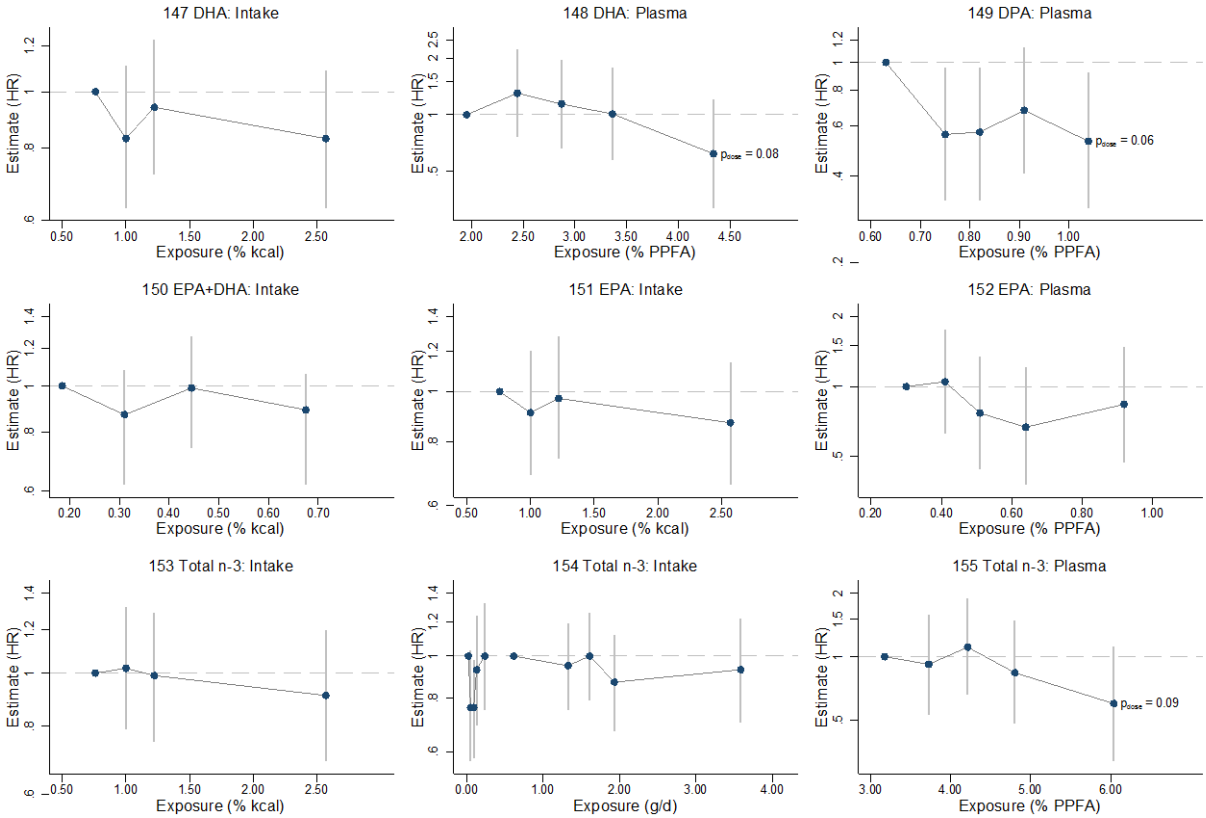
Observational study subgroup analyses

The Shanghai study found no significant difference in association (of total n-3 FA intake) by baseline total cholesterol to HDL-c ratio.[164]

Table G.1. Total Stroke Death: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs Placebo											
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	≥0.85 g/d (marine oil) [E:D 0.9:1-1.5:1]	Placebo	0 (Olive oil)	5 y	Self-reported (nd on level of adherence)	19/6239, 0.3%	18/6266, 0.3%	HR 1.05 (0.55, 2.00)	0.88
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	0.850-0.882 g/d (marine oil) [E:D 1:1.2]	Placebo	0 (nd)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	50/3494, 1.4%	44/3481, 1.3%	OR 1.13 (0.75, 1.71)	
Burr 1989 2571009 UK	CVD	Fish advice, either alone or in combination with fiber advice, fat advice, or both fiber and fat advice.	EPA 0.34 g/d (diet)	No fish advice (Fat advice, fiber advice, fiber and fat advice, or no advice)	EPA 0.09 g/day (diet)	>10 y	Compliance was good based on dietary assessments	29/1015, 2.9%	23/1018, 2.3%	OR 1.23 (0.71, 2.14)	NS

Figure G.4. n-3 FA associations with total stroke death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles.
Blue circles = healthy adults.

Ischemic Stroke Death

Randomized Controlled Trials

No RCTs evaluated this outcome.

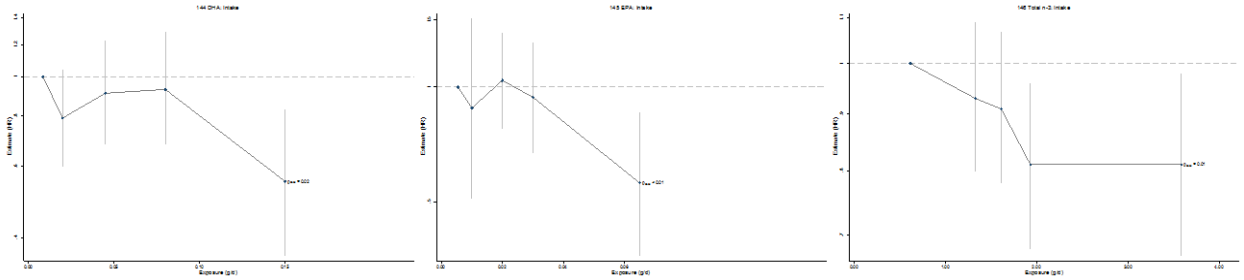
Observational Studies

Two studies evaluated the association between n-3 FA intake and risk of ischemic stroke death in healthy adults (**Appendix Table H.3, Figure H.4**).[164, 185] Both found significant associations. JACC found an association between higher intake of total n-3 FA (combined) and lower risk of ischemic stroke death after about 13 years of followup (plot #146), with significant associations found in quintiles with median intake of about 2 g/d or more.[185] The Shanghai Women's and Men's Health Studies found similar significant associations with higher EPA (particularly for median intake >0.07 g/d in men an d>0.06 g/d in women), DHA (particularly for median intake >0.15 g/d), and combined EPA+DHA intake (in separate analyses) with about 11 years of followup in women and 6 years of followup in men (plots #144 & 145; EPA+DHA not plotted because no data were provided for median intake per quantile).[164]

Observational study subgroup analyses

The Shanghai study found no significant difference in association (of total n-3 FA intake) by baseline total cholesterol to HDL-c ratio.[164]

Figure H.4. n-3 FA associations with ischemic stroke death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for ischemic stroke death. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles.
Blue circles = healthy adults.

Hemorrhagic Stroke Death

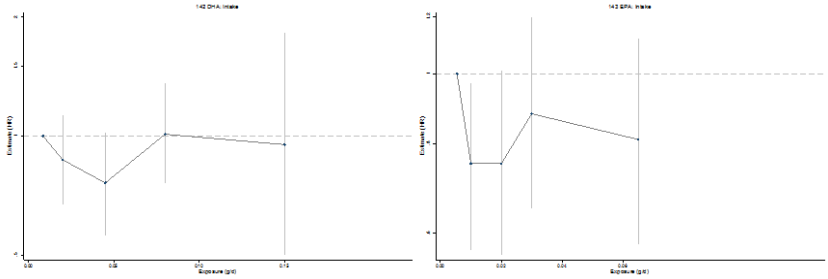
Randomized Controlled Trials

No RCTs evaluated this outcome.

Observational Studies

Only the Shanghai Women's and Men's Health Studies evaluated hemorrhagic stroke death (**Appendix Table J.3, Figure J.3**).[164] The study found no association between EPA, DHA, and EPA+DHA intake (not graphed because no data on median intake per quantile), separately, and risk of hemorrhagic stroke death after about 11 years followup in women and 6 years followup in men (combined analyses).

Figure J.3. n-3 FA associations with hemorrhagic stroke death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome.
Blue circles = healthy adults.

Death, All-Cause

Randomized Controlled Trials

Sixteen RCTs evaluated all-cause death (**Table K.1**).[56, 59, 61, 62, 78, 79, 88, 114, 116, 123, 126, 137, 141, 150, 153, 168] Of these, one study was conducted in 12,716 generally healthy participants,[137] two were in a total of 13,068 participants at risk of CVD (defined as hypercholesterolemia,[78] or a combination of various risk factors[153]), and 13 in a total of 49,578 participants with CVD including previous persistent AFib,[123] DM or a history of CVD,[56] arrhythmia,[59, 116] CAD,[79] all CVD,[88] MI,[62, 114, 126, 141, 150] heart failure,[168] and angina.[61]

Marine oil vs. placebo

Meta-analysis of the 15 RCTs of marine oil versus placebo yielded a nonsignificant summary effect size for risk of all-cause death: HR=0.97 (95% CI 0.91, 1.04) (**Figure K.2**).

At risk for CVD population

Among 13,068 participants at risk of CVD, two RCTs compared marine oil (EPA+DHA) with placebo (corn or olive oil) (**Figure K.2**).[78, 153] The doses of EPA+DHA were greater than 0.85 and 2.02 g/d, with EPA to DHA ratio ranging from 0.9 to 1.5. Compliance was greater than 90% in one study and was not reported in another. The duration of followup was 3 and 5 years. Both RCTs found that EPA+DHA had no significant effect on all-cause death compared with placebo (adjusted HR 0.53, 95% CI 0.27, 1.04; HR 1.03, 95% CI 0.88, 1.19).

Subgroup meta-analysis (as part of a meta-analysis of all marine oil vs. placebo trials) yielded a summary HR of 0.98 (95% CI 0.80, 1.21).

CVD population

Among the 13 RCTs that were conducted in participants with CVD (**Figure K.2**), eight studies compared marine oil (EPA+DHA) with placebo,[56, 59, 88, 116, 123, 141, 150, 168] two compared marine oil (EPA+DHA) with no intervention,[79, 126] two compared "fish advice" (advise to increase fish intake in both studies with additional advise to take fish oil supplement in later study) with "no fish advice",[61, 62] and one was the 2-by-2 factorial RCT described under *Major Adverse Cardiovascular Events* that compared EPA+DHA, EPA+DHA and ALA, ALA, and oleic acid margarines.[114]

Among the 11 studies compared marine oil (EPA+DHA) with placebo or no intervention, a total of 44,431 participants with CVD were examined [56, 59, 79, 88, 114, 116, 123, 126, 141, 150, 168] The doses of EPA+DHA ranged from 0.4 g/d to 3.32 g/d. Among the 8 RCTs reporting sufficient detail, the EPA to DHA ratio ranged from 0.5 to 2. Compliance ranged from 65 to 88 percent. The duration of follow-up ranged from 1 year to more than 6 years. Two of the 11 RCTs found that EPA+DHA had significant effect on reducing all-cause death compared with placebo or no intervention in 6975 participants with heart failure (adjusted HR 0.91; 95% CI 0.833, 0.998) and in 11,332 participants with MI (RR 0.79; 95% CI 0.66, 0.93). The other nine RCTs found that EPA+DHA did not have significant effect on all-cause death with OR/HR ranging from 0.52 to 1.25.

Among the two studies that compared "fish advice" with "no fish advice",[61, 62] a total of 5147 participant with MI or angina were examined. The mean EPA intake estimated by the dietary assessment was 0.34 and 0.45 g/d in the "fish advice" groups, and was 0.09 and 0.11 in the "no fish advice" groups. No estimates for DHA intake levels were reported. Compliance was

good (fish intake was significantly increased in the "fish advice" groups) based on the dietary assessments. Both RCTs found no significant difference in the risk of all-cause death between groups (HR 0.95; 95% CI 0.85, 1.07; HR 1.15, 95% CI 0.92, 1.32).

Across the 13 RCTs of CVD populations, the summary HR (**Figure K.2**) was 0.97 (0.90, 1.05); almost identical to the nonsignificant summary HR for all RCTs, regardless of population (HR 0.97; 95% CI 0.91, 1.04).

ALA vs. placebo

Healthy population

Among 12,716 healthy people, one RCT compared ALA oil (linseed oil) to control oil (sunflower seed oil).[137] The doses of ALA were 5.2 and 0.13 g/d, respectively. Compliance was not reported. After 1-year followup, there was no significant difference in all-cause death between the two groups (OR 0.93; 95% CI 0.61, 1.44).

CVD population

Among 4837 participants with MI, the 2-by-2 factorial RCT found no significant difference in the risk of all-cause death compared with the groups received EPA+DHA alone or placebo margarines (HR 0.97; 95% CI 0.79, 1.19).[114]

RCT subgroup analyses

Four RCTs included subgroup analysis for all causes of death (**Table K.3**). All trials compared marine oil against placebo. One trial found no significant difference in effect between patients with and without hypertension (P interaction = 0.67).[126] Among the two analyses of diabetes vs no diabetes subgroups neither reported a statistically significant interaction between diabetes and marine oils.[126, 168] One study found no interactions between marine oil and age, left ventricular ejection fraction, ischemic cause vs. nonischemic cause of existing CVD, New York Heart Association level, total cholesterol, or statin use. A third study found no significant difference in effect regardless of B vitamin supplemention.[88] The fourth study found no difference in effect between patients with history of CVD compared to patients without a history of CVD.[78]

Meta-regression of the marine oil trials found no significant interaction between n-3 FA dose (P=0.45), followup time (P=0.64), or between at risk and CVD populations (P=0.65)

Observational Studies

Seven studies evaluated the associations between n-3 FA intake or biomarker levels and all-cause death, mostly in healthy adults after 7 to 30 years of followup (**Appendix Table K.3, Figure K.4**); one study evaluated CVD patients with a history of MI after 4 years of followup.[75, 85, 96, 117, 134, 136, 164, 179, 185] Most analyses found significant associations between higher n-3 FA intake or biomarker level and reduced risk of death.

n-3 FA Intake

Five studies evaluated n-3 FA intake and the risk of death (Cardiovascular Health Study, JACC, MRFIT, Shanghai Women's and Men's Health Studies [two separate studies analyzed together], Takayama).[75, 134, 136, 164, 185]

JACC found no association between total n-3 FA intake (combined) and all-cause death in healthy adults after about 13 years of followup (plot #106).[185]

Two studies evaluated ALA intake. MRFIT and the Cardiovascular Health Study both found significant associations between higher ALA intake and reduced death in healthy men after about 10 years and healthy adults ≥65 years old after 12 years (plots # 96 & 97), with significant or larger associations found in median quantiles with intakes above about 1.6 g/d, 1 percent Kcal, or 2.4 percent of fat intake.[75, 134]

In a combined analysis (of women and men), the Shanghai Women's and Men's Health Studies found a significant associations between higher EPA and DHA intakes (separately) and reduced death after about 11 years of followup in the women and 6 years of followup in the men (plots #99 & 104), with significant associations found for quintiles with median intakes above 0.01 g/d of EPA and above 0.02 g/d of DHA.[164]

Three studies found heterogeneous associations between EPA+DHA (or EPA+DHA+DPA) intake and death risk (plots #102 & 103). MRFIT found nonsignificant associations between higher marine oil intake and death after 10 years of followup ($P<0.10$).[75] The Takayama study found no association in healthy men, but significantly lower death among women with higher marine oil intake after 7 years of followup.[136] The combined Shanghai Women's and Men's Health Studies found a significant associations between higher marine oil intake and lower risk of death in women after 11 years of followup and men after 6 years of followup.[164] Across studies, associations were large or near-significant in quantiles with median intake above about 0.3 percent Kcal or about 0.7 or 1.2 g/d.

n-3 FA Biomarkers

Three studies evaluated associations between n-3 FA biomarkers and risk of death, two in healthy adults, one in CVD patients with a history of MI.

The Cardiovascular Health Study found a significant association between higher plasma n-3 FA levels (combined) and risk of death in healthy adults ≥65 years after 16 years of followup (plot #107).[117]

Two studies evaluated ALA biomarkers (plot #98). The Cardiovascular Health Study and ULSAM found no significant associations between plasma ALA and risk of death at 16 and 31 years of followup in healthy adults.[117, 179]

Three studies evaluated EPA biomarkers (plot #105), one in a CVD population. The Osaka Acute Coronary Insufficiency Study found no association between blood EPA levels and death in patients with a history of MI after 4 years of followup. Similarly, ULSAM found no association with plasma EPA after 31 years of followup.[179] In contrast, the Cardiovascular Health Study found a significantly lower risk of death with higher plasma EPA levels after 16 years of followup in healthy adults ≥65 years old.[117]

The same three studies evaluated DHA biomarkers (plot #100). In contrast with its finding regarding blood EPA levels, the Osaka Acute Coronary Insufficiency Study found a significant association between higher blood DHA levels and reduced death. In ULSAM and the Cardiovascular Health Study, findings were concordant between blood EPA and DHA levels, such that the former found no association with death and the latter found a significant association between higher plasma DHA levels and lower death.[117, 179]

The Cardiovascular Health Study also found a significant association between higher plasma DPA levels and lower all-cause death in healthy adults (plot #101).

Observational study subgroup analyses

Three observational studies conducted subgroup analyses of the associations between n-3 FA and all-cause death (**Table K.5**). The Takayama study implied no difference in association of EPA+DHA intake between men and women.[136] The Cardiovascular Health Study found no difference in association of intake of or plasma ALA based on baseline fish consumption.[117] The Osaka Acute Coronary Insufficiency Study evaluated 12 sets of subgroups for both blood DHA and blood EPA, as listed in Table K.5. A statistically significant interaction was found between blood EPA and hypertension (P interaction = 0.015). In participants with hypertension, no association was found between blood EPA and risk of death (HR=0.96); however, in participants with no hypertension, higher blood EPA was associated with higher risk of dying (HR=8.23). The study also found near significant interactions between blood EPA and diabetes (P interaction = 0.089, favoring those without diabetes) and statin use (P interaction = 0.062, favoring those not using statins).

Table K.1. All-cause death: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo											
Einvik 2010 20389249 Norway	At Risk	EPA+DHA+diet intervention	2.02 g/d (Marine oil) [E:D 1.4]	Placebo+diet intervention	0 (Corn oil))	3 y	>90% of the tablets were taken based on pharmacy records, and verified by biomarkers	14/282, 4.96%	24/281, 8.54%	Adj HR 0.53 (0.27, 1.04)	0.063
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	≥0.85 g/d (suppl) [E:D 0.9-1.5]	Placebo	0 (Olive oil)	5 y	Self-reported (nd on level of adherence)	348/6239, 5.6%	337/6266, 5.4%	HR 1.03 (0.88, 1.19)	0.73
Macchia 2013 23265344 Argentina and Italy	CVD	EPA+DHA	0.85-0.882 (suppl) [nd]	Placebo	0 (Olive oil)	12 mo	nd	4/289, 1.4%	5/297, 1.7%	HR 0.80 (0.21, 3.00)	NS
Bosch 2012 22686415 Canada	CVD[d]	EPA+DHA	0.84 g/d (Marine oil) [E:D 1.24]	Placebo	0 (Olive oil)	6+ y	Followup (adherence was 88% at the end of study)	951/6281, 15.1%	964/6255, 15.4%	Adj HR 0.98 (0.89, 1.07)	0.63
Brouwer 2006 16772624 N Europe	CVD	EPA+DHA	0.96g n-3 PUFAs (0.464 g EPA, 0.335g DHA) (Marine oil) [E:D=1.4]	Placebo	0 (high-oleic acid sunflower oil)	1 y	Generally good (76% reported taking 80% pills) based on pill counts and confirmed by biomarkers.	8/273, 3%	15/273, 5%	OR 0.52 (0.22, 1.25)	0.142
Leaf 2005 16267249 US	CVD	EPA+DHA	EPA plus DHA of 2.6 g (Marine oil)	Placebo	0 (Olive oil)	12 mo	Pill counts and analysis of the phospholipids of red blood cells for their content of EPA and DHA. Noncompliance ~35%	13/200, 6.5%	12/202, 5.9%	OR 1.10 (0.49, 2.47)	0.816

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Galan 2010 21115589 France	CVD	EPA+DHA	EPA 0.4 g/d DHA 0.2g/d (Marine oil) [E:D=2]	Placebo	0 (nd)	4.7 y	Patient reported (86% reported they took >=80% of allocated treatment)	58/1253, 4.7%	59/1248, 4.7%	Adj HR 1.03 (0.72, 1.48)	0.88
Nilsen 2001 2001 11451717 Norway	CVD	EPA+DHA	EPA-DHA 3.4-3.528 g/d (Marine oil) [E:D=0.5]	Placebo	0 (Corn oil)	29 mo (median)	82% in fish oil group; 86% in the placebo group	21/150, 14%	18/150, 12%	OR 1.19 (0.61, 2.34)	0.607
Rauch 2010 21060071 Germany	CVD	EPA+DHA	0.46g EPA, 0.38g DHA (Marine oil) [E:D=1.2]	Placebo	0 (Olive oil)	1 y	Pill counts at 3 months and 12 months (≥70% of study period)	88/1919, 4.6%	70/1885, 3.7%	OR 1.25 (0.90, 1.72)	0.18
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine oil) [E:D 0.83]	Placebo	0 (nd)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	955/3494, 27.3%	1014/3481, 29.1%	Adj HR 0.91 (0.833, 0.998)	0.041
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA (±ALA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±ALA)	0; 2 g/d ALA (Placebo margarine = oleic acid; Plant oil)	40 mo	90% of the patients adhered fully to the protocol; verified by biomarkers	186/2404, 7.7%	184/2433, 7.6%	HR 1.01 (0.82, 1.24)	0.92
Eritsland 1996 8540453 Norway	CVD	EPA+DHA	EPA 2.04 g/d, DHA 1.28 g/d (Marine oil) [E:D=1.6]	No intervention	0	1 y	Tablet and capsule accounts (88% were taken), and serum phospholipid fatty acids	8/317, 2.5%	6/293, 2.0%	OR 1.24 (0.42, 3.61)	0.695
Marchioli 2002 11997274 Italy	CVD	EPA+DHA	0.850- 0.882 g/d (Marine oil) [E:D 0.5]	No intervention	nd	42 mo	Followup (adherence was 72.5% at the end of study)	477/5679, 8.4%	554/5653, 9.8%	RR 0.79 (0.66, 0.93)	0.0006
Burr 1989 2571009 UK	CVD	Fish advice, either alone or in combination with fiber advice, fat advice, or both fiber and fat advice.	EPA 0.34 g/d (diet)	No fish advice (Fat advice, fiber advice, fiber and fat advice, or no advice)	EPA 0.09 g/day (diet)	Overall years (10+ y)	Compliance was good based on dietary assessments	530/1015, 52.2%	553/1018, 54.3%	Adj HR 0.95 (0.85, 1.07)	NS

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Burr 2003 12571649 UK	CVD	Fish advice, fish+fish oil	EPA 0.45 g/d (diet)	No fish advice	EPA 0.11 (diet)	9 y	Dietary charts sent by post with reply-paid envelopes	283/1571, 18.0%	242/1543, 15.7%	Adj HR 1.15 (0.92, 1.36)	0.13
ALA vs. Placebo											
Natvig 1965 5756076 Norway	Healthy	ALA	ALA 5.2 g/d (Linseed oil)	Control oil	ALA 0.13 g/d (Sunflower seed oil)	1 y	nd	40/6690, 6%	43/6716, 6%	OR 0.93 (0.61, 1.44)	0.755
Kromhout 2010 20929341 Netherlands	CVD	ALA (±EPA+DHA)	0.4 g/d EPA+DHA and 2 g/d ALA (Marine; Plant oil) [E:D 3:2]	Placebo (±EPA+DHA)	0; 0.4 g/d EPA-DHA (placebo = oleic acid; Marine oil) [E:D 3:2]	40 mo	90% of the patients adhered fully to the protocol; verified by biomarkers	182/2404, 7.6%	188/2433, 7.7%	HR 0.97 (0.79, 1.19)	0.8

[d] DM and history of CVD

Figure K.2. All-cause death: Randomized trials of marine oils

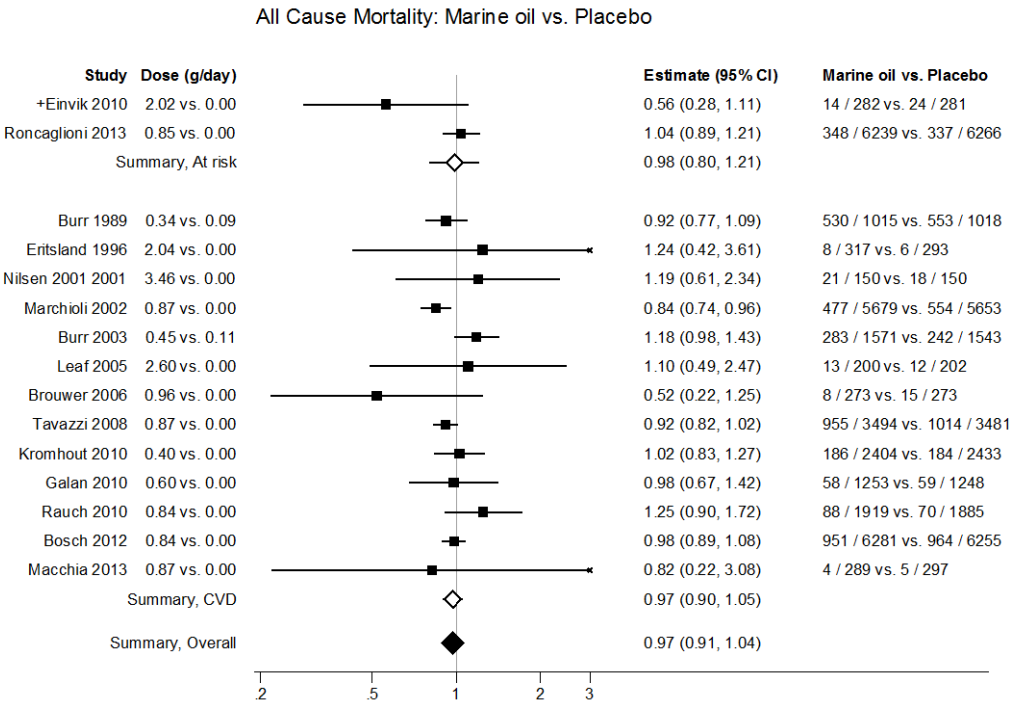
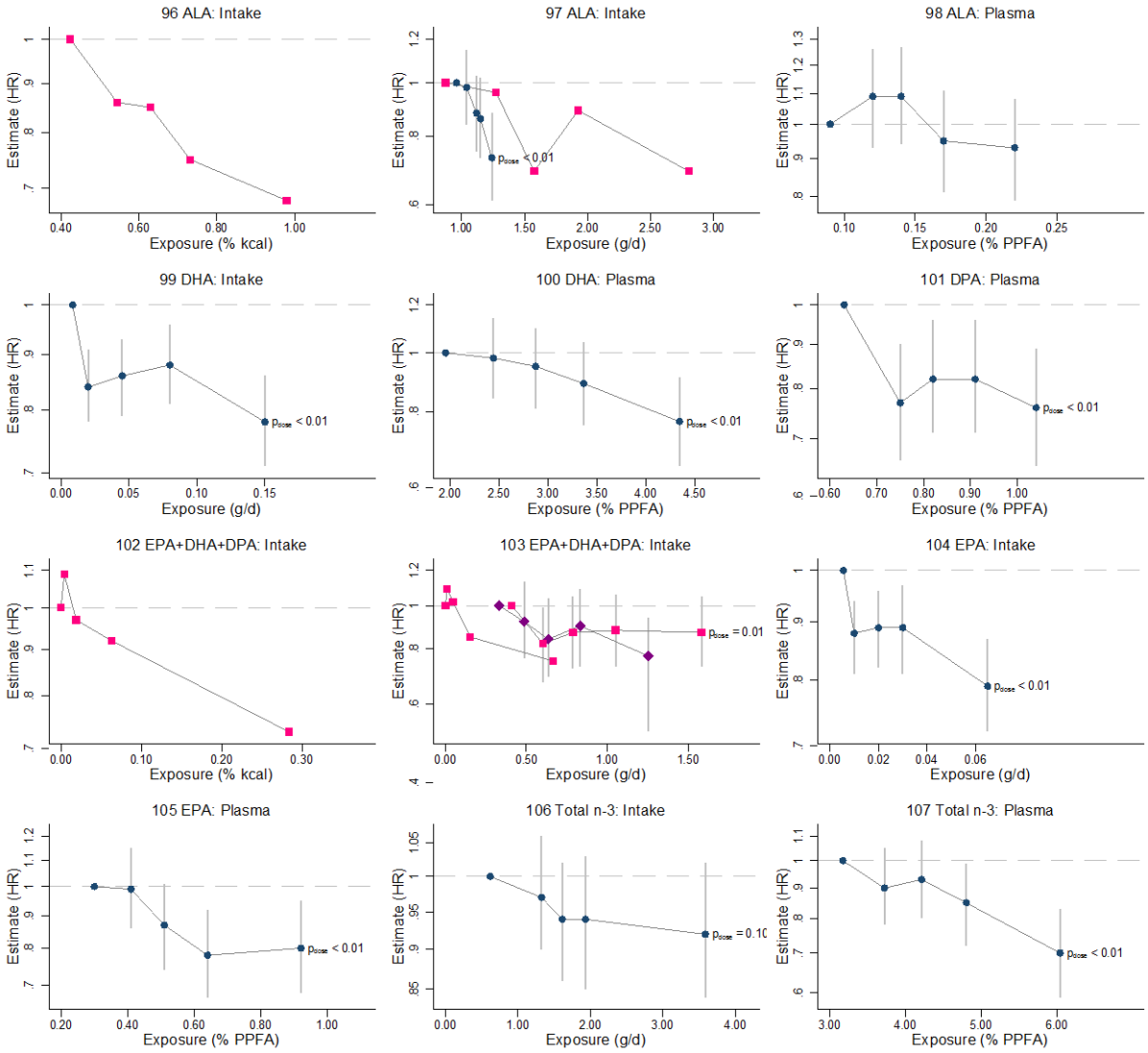


Table K.3. All-cause death: Subgroup Analyses, Randomized trials

Study	Population	Subgroups	n-3 FA	Comparator	N Total	P difference	Difference	Favors
Marchioli 2002 11997274 Italy	CVD	HTN vs no HTN	EPA+DHA	Placebo	11323	0.67		
		Diabetes vs no diabetes	EPA+DHA	Placebo	11323	0.50		
Tavazzi 2008 18757090 Italy	CVD	Diabetes vs no diabetes	EPA+DHA	Placebo	6975	NS		
		Age <69 vs ≥69 years	EPA+DHA	Placebo	6975	NS		
		Left ventricular ejection fraction ≤40% vs >40%	EPA+DHA	Placebo	6975	NS		
		Ischemic cause vs nonischemic cause	EPA+DHA	Placebo	6975	NS		
		New York Heart Association II vs III or IV	EPA+DHA	Placebo	6975	NS		
		Total cholesterol ≤4.87 vs >4.87 mmoL/l	EPA+DHA	Placebo	6975	NS		
		With statin vs without statin	EPA+DHA	Placebo	6975	NS		
Galan 2010 21115589 France	CVD	B vitamin vs no B vitamin	EPA+DHA	Placebo	2501	NS		
Einvik 2010 20389249 Norway	At Risk	With history of CVD vs no history of CVD	EPA+DHA	Placebo	563	NS		

Figure K.4. n-3 FA associations with all-cause death: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles. Where 95% confidence intervals (vertical lines) are missing, these were not reported in the studies.
Blue circles = healthy adults, pink squares = healthy males, purple diamonds = healthy females.

Table K.5. All-cause death: Subgroup Analyses, Observational studies

Study	Subgroups	n-3 FA	N Total	P difference	Difference	Favors
Takayama[136]	Men vs Women	EPA+DHA intake	30480	NS (implied)		
Cardiovascular Health Study[117]	Fish consumption vs low or no fish consumption	ALA (Plasma or Intake)	4432	NS		
	Men vs Women			NS		
Osaka Acute Coronary Insufficiency Study[96]	Age <65 vs ≥65 years	DHA (Blood)	671	0.63		
	Male vs Female			0.83		
	Diabetes vs. no diabetes			0.21		
	Hypertension vs. no hypertension			0.30		
	Dyslipidemia vs. no dyslipidemia			0.31		
	LDL-c <100 vs ≥100 mg/dL			0.80		
	HDL-c <40 vs ≥40 mg/dL			0.81		
	Tg <150 vs. ≥ 150 mg/dL			0.56		
	eGFR <60 vs. ≥60 mL/min			0.69		
	Statin vs no statin			0.31		
	ACEi/ARB vs. no ACEi/ARB			0.40		
	Beta blocker vs. no beta blocker			0.77		
	Age <65 vs ≥65 years	EPA (Blood)	671	0.15		
	Male vs Female			0.24		
	Diabetes vs. no diabetes			0.089	HR 2.73 vs. 0.92	No diabetes
	Hypertension vs. no hypertension			0.015	HR 0.96 vs. 8.23	Hypertension
	Dyslipidemia vs. no dyslipidemia			0.44	nd	
	LDL-c <100 vs ≥100 mg/dL			0.74	nd	
	HDL-c <40 vs ≥40 mg/dL			0.94	nd	
	Tg <150 vs. ≥ 150 mg/dL			0.56	nd	
	eGFR <60 vs. ≥60 mL/min			0.38	nd	
	Statin vs no statin			0.062	HR 2.64 vs. 0.83	No statin
	ACEi/ARB vs. no ACEi/ARB			0.97	nd	
	Beta blocker vs. no beta blocker			0.72	nd	

Coronary Heart Disease, Incident

Randomized Controlled Trials

No RCT evaluated incident coronary heart disease.

Observational Studies

Eleven studies evaluated the associations between intake and biomarkers of n-3 FA and incident coronary heart disease (CHD) (**Appendix Table L.3, Figure L.4**).[48, 49, 69, 72, 85, 102, 104, 112, 117, 134, 147, 172] . Definitions of CHD outcomes varied across studies, but mostly included both fatal and nonfatal events. All studies were conducted in generally healthy adults. The median followup duration across studies was 11.5 years (range of average followup 6 to 23 years). Studies found a mix of both significant associations between higher n-3 FA intake or biomarker levels and lower risk of CHD or a lack of associations.

n-3 FA Intake

Ten studies evaluated n-3 FA intake and risk of CHD (Alpha-Tocopherol, Beta-Carotene Cancer Prevention, Cardiovascular Health Study, Glostrup Population Studies, Health Professional Follow-up Study, Japan Public Health Center-Based Study - Cohort I, MESA, MORGEN, Nurses' Health Study, Pooling Project of Cohort Studies on Diet and Coronary Disease, Spanish EPIC).

Six studies evaluated ALA intake with 6 to 23 years of followup (Pooling Project of Cohort Studies on Diet and Coronary Disease, Alpha-Tocopherol Beta-Carotene Cancer Prevention, Cardiovascular Health Study, Glostrup Population Studies, MESA, MORGEN). One of these studies, the Pooling Project, pooled data from eight large cohorts (ARIC, FMC, IWHS, NHS, VIP, WHS, ATBC, HPFS); thus, overall 13 study cohorts were included (plot #29). Individually, none of the studies found associations between ALA intake and CHD.

By meta-analysis (**Table L.6**), overall there is no association between ALA intake and CHD across a median dosage range of 0.45 to 2.5 g/d (effect size per g/d = 0.99 [95% CI 0.93, 1.05]). Meta-analyses with the addition of a spline knot point (from 0.5 to 1.4 g/d) found a best-fit curve with a change in slope (between g/d and risk of CHD) at 0.5 g/d, but both above and below this threshold the associations between intake and CHD were nonsignificant (<0.5 g/d: effect size per g/d = 0.87 [95% CI 0.67, 1.13]; >0.5 g/d: effect size per g/d = 1.03 [95% CI 0.93, 1.15]). Analyses at all thresholds between 0.5 and 1.4 g/d gave similar results.

For both EPA and DHA, separately, two studies evaluated associations with CHD, both at about 10 years of followup (plots #32 & 41). Spanish EPIC found no associations between DHA or EPA intake and CHD in either men or women (analyzed separately).[48] MESA found a near-significant possible associations (P=0.09 DHA and 0.06 EPA) between higher DHA and EPA intake and lower risk of CHD.[72]

Only MESA evaluated DPA intake, finding significantly lower risk of CHD among those with higher DPA intake after 10 years of followup (plot #35).[72]

Table L.6. Meta-analysis results of observational studies of ALA intake and CHD

N Patients	Dose Range, g/d	Knot	Effect Size (ES), Overall	ES below knot	ES above knot	AIC	No. cohorts crossing threshold
50,231	0.45-2.5	NA	0.99 (0.93, 1.05)			−14.2	6
		0.5		0.87 (0.67, 1.13)	1.03 (0.93, 1.15)	16.8	6
		0.6		0.91 (0.74, 1.13)	1.02 (0.92, 1.14)	17.0	6
		0.7		0.94 (0.79, 1.12)	1.02 (0.91, 1.13)	20.8	6
		0.8		0.95 (0.82, 1.10)	1.02 (0.91, 1.14)	19.5	5
		0.9		0.95 (0.84, 1.09)	1.02 (0.91, 1.14)	19.0	5
		1.0		0.96 (0.85, 1.07)	1.02 (0.91, 1.14)	18.6	5
		1.1		0.96 (0.87, 1.07)	1.02 (0.91, 1.15)	19.5	5
		1.2		0.97 (0.88, 1.06)	1.02 (0.91, 1.15)	24.6	5
		1.3		0.97 (0.89, 1.06)	1.02 (0.90, 1.16)	29.0	4
		1.4		0.97 (0.90, 1.06)	1.02 (0.89, 1.17)	31.5	4

Seven studies evaluated intake of EPA+DHA (five studies) or EPA+DHA+DPA (two studies) with 6 to 23 years of followup (Alpha-Tocopherol Beta-Carotene Cancer Prevention, Glostrup Population Studies, Health Professional Follow-up Study, Japan Public Health Center-Based Study - Cohort I, MESA, Nurses' Health Study, Spanish EPIC) (plots #38 & 39). Individually, studies found variable associations. In two analyses of combined men and women (Japan Public Health Center-Based Study - Cohort I, MESA), neither found a significant association at 10 and 11.5 years of followup (although, MESA found a lower risk with higher EPA+DHA+DPA intake at P=0.08).[72, 104] Three studies analyzed associations in women specifically (Glostrup Population Studies, Nurses' Health Study, Spanish EPIC). The Nurses' Health Study and Glostrup Population Studies found significantly lower risk of CHD with higher EPA+DHA intake[102, 172] ; the Spanish EPIC study also found lower HRs with higher intake but the association was nonsignificant.[48] Four studies analyzed men specifically (Alpha-Tocopherol Beta-Carotene Cancer Prevention, Glostrup Population Studies, Health Professional Follow-up Study, Spanish EPIC). All found no significant associations; however, in contrast with the studies of all adults or of women, the direction of the associations suggested *higher* risk of CHD among men with higher marine oil intake at baseline.[48, 49, 147, 172] By meta-analysis (**Table L.7**), overall there is a near significant association between marine oil intake and CHD across a median dose range of 0.038 to 3.47 g/d (effect size per g/d = 0.90 [95% CI 0.80, 1.01]). Meta-analyses with the addition of a spline knot point (from 0.1 to 1.4 g/d) found a best-fit curve with a change in slope (between g/d and risk of CHD) at 1.0 g/d. Below this threshold, increasing dose of marine oil was protective against CHD (effect size per g/d = 0.77 [95% CI 0.65, 0.91]); above marine oil intake of 1.0 g/d, there is no significant association (effect size per g/d = 1.08 [95% CI 0.87, 1.35]). However, similar results are found with thresholds from 0.2 to 1.4 g/d.

Table L.7. Meta-analysis results of observational studies of marine oil intake and CHD

N Patients	Dose Range, g/d	Knot	Effect Size (ES), Overall	ES below knot	ES above knot	AIC	No. cohorts crossing threshold
155,143	0.038-3.47	NA	0.90 (0.80, 1.01)	NA	NA	−10.9	8
		0.1		0.29 (0.08, 1.02)	0.97 (0.85, 1.10)	39.5	8
		0.2		0.47 (0.24, 0.92)	0.98 (0.86, 1.11)	31.9	8
		0.3		0.60 (0.39, 0.94)	0.98 (0.86, 1.12)	33.3	8
		0.4		0.65 (0.46, 0.93)	0.99 (0.87, 1.14)	38.0	7
		0.5		0.68 (0.51, 0.91)	1.01 (0.87, 1.17)	35.3	6
		0.6		0.70 (0.55, 0.90)	1.02 (0.88, 1.20)	33.3	6
		0.7		0.72 (0.57, 0.90)	1.04 (0.88, 1.24)	32.0	6
		0.8		0.74 (0.60, 0.90)	1.06 (0.88, 1.28)	30.9	6
		0.9		0.76 (0.63, 0.91)	1.07 (0.87, 1.30)	30.0	6
		1.0		0.77 (0.65, 0.91)	1.08 (0.87, 1.35)	29.6	6
		1.1		0.78 (0.67, 0.92)	1.10 (0.87, 1.40)	30.1	6
		1.2		0.80 (0.69, 0.92)	1.11 (0.86, 1.44)	30.7	6
		1.3		0.81 (0.71, 0.93)	1.11 (0.84, 1.48)	31.7	6
		1.4		0.81 (0.71, 0.93)	1.15 (0.83, 1.59)	34.5	6

n-3 FA Biomarkers

Three studies analyzed n-3 FA biomarkers (Cardiovascular Health Study, EPIC Norfolk, MESA) in healthy adults (men and women combined) with 10, 13, and 16 years of followup.[72, 112, 134]

The two studies that evaluated blood or plasma levels of total n-3 FA combined had conflicting findings regarding the association between total n-3 FA biomarkers and risk of CHD (plots #44 & 45). EPIC Norfolk found no evidence of an association between blood levels of total n-3 FA and risk of CHD at 13 years,[112] but the Cardiovascular Health Study found a significantly lower risk of CHD at 16 years with higher total n-3 FA plasma levels.[117, 134]

All three studies (Cardiovascular Health Study, EPIC Norfolk, MESA) found no association between ALA blood, plasma, or phospholipid levels and risk of CHD (plots #30 & 31).[72, 112, 134]

All three studies evaluated both EPA and DHA blood, plasma, or phospholipid levels (separately for each n-3 FA) and found similar associations for the two n-3 FA (plots #33, 34, 42, & 43). The Cardiovascular Health Study and MESA both found lower risk of CHD associated with higher baseline EPA and DHA levels.[72, 117, 134] EPIC Norfolk found no association.[112]

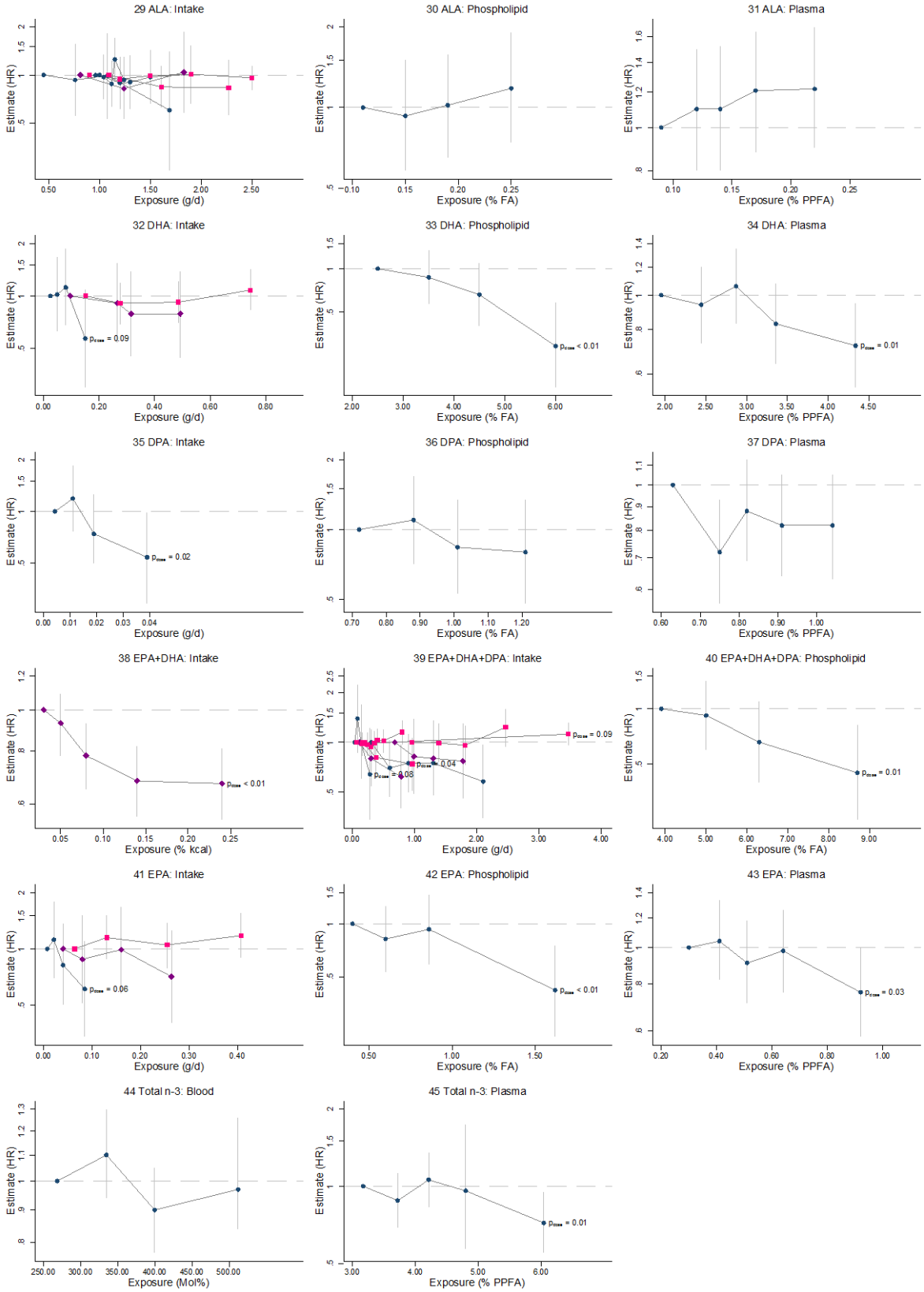
The three studies also evaluated DPA blood, plasma, or phospholipid levels, but each study had the opposite findings as for EPA and DHA biomarkers (plots #36 & 37). The Cardiovascular Health Study and MESA found no significant association with CHD (although the HR estimates also favored lower CHD with higher DPA levels).[72, 117, 134] EPIC Norfolk found a significantly lower risk of CHD with higher DPA blood levels.[112]

The MESA study found a significant association between combined EPA+DHA+DPA phospholipid levels and lower risk of CHD (plot #40).[72]

Observational study subgroup analyses

The Pooling Project found a stronger, almost significant, association between ALA intake and incident CHD in men (HR=0.85; 95% CI 0.72, 1.01) than in women (HR=1.02; 95% CI 0.65, 1.59), but did not report whether these associations were significantly different from each other (whether there was an interaction).[173] The Cardiovascular Health Study found no difference in associations between ALA (plasma or intake) and incident CHD between men and women or between those with higher versus lower (or no) fish intake at baseline.[117]

Figure L.4. n-3 FA associations with incident coronary heart disease: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles.
Blue circles = healthy adults, black circles = adults with dyslipidemia (at risk), pink squares = healthy males, purple diamonds = healthy females.

Myocardial Infarction, Total (Fatal and Nonfatal)

Randomized Controlled Trials

Nine RCTs evaluated risk of myocardial infarction (MI) (**Table M.1**).[56, 59, 62, 88, 137, 153, 155, 168, 187] Of these, one study was conducted in 12,716 generally healthy participants,[137] three were in a total of 27,938 participants at risk of CVD (defined as previous stable angina,[137] dyslipidemia,[187] or a combination of various risk factors,[153] and seven were in a total of 28,906 participants with CVD including MI,[62, 137, 153] CAD,[155, 187] DM and history of CVD,[56] all CVD,[88] heart failure,[168] previous persistent AFib,[123] and arrhythmia.[59] One of the marine oil trials reported separate analyses for at risk and CVD populations.[187] One of the ALA trials reported separate analyses for all three population groups.[137]

Marine oil vs. placebo

At risk for CVD population

Two RCTs comparing marine oils to control were conducted in participants at increased risk of CVD.[153, 187] One compared 1.8 g/d EPA combined with statin with control (statin alone) in 14,981 participants with dyslipidemia (without CAD),[187] and one compared marine oil (EPA+DHA) with placebo (olive oil) in 12,505 participants with a combination of various risk factors.[153] Compliance was not reported in either study. After 5-year followup, the EPA (and statin) study showed no significant additive effect of EPA on statin use to reduce the risk of MI compared with statin alone (HR 0.79; 95% CI 0.52, 1.19). In the RCT of EPA+DHA,[153] the dose of EPA+DHA was less than 0.85 g/d with a EPA to DHA ratio between 0.9 and 1.5. After 5-year followup, this study found that EPA+DHA had no significant effect on risk of MI compared with placebo (HR 0.76; 95% CI 0.34, 1.74)

Subgroup meta-analysis (as part of a meta-analysis of all marine oil vs. placebo trials) yielded a nonsignificant summary HR of 0.78 (95% CI 0.52, 1.17).

CVD population

Seven RCTs of participants with a history of CVD evaluated EPA+DHA supplementation or fish advice to placebo (or no fish advice) in a total of 28,314 participants.[56, 59, 62, 88, 155, 168, 187] Followup duration ranged from 1 to over 6 years. Among the six EPA+DHA trials, total dose of marine oil ranged from 0.6 to 6 g/d; the fish advice trial compared 0.34 g/d (based on food frequency questionnaire) to 0.09 g/d. Among five of the RCTs, the ratio of EPA to DHA ranged from 0.83 to 1.4. None of the trials found a statistically significant effect of marine oil on risk of MI, with effect sizes ranging from 0.43 (95% CI 0.04, 5.06) to 1.09 (95% CI 0.93, 1.27).

Across the seven RCTs of CVD populations, the summary HR (**Figure M.2**) was 0.91 (0.78, 1.06); almost identical to the near-significant summary HR for all RCTs, regardless of population (HR 0.89; 95% CI 0.77, 1.03).

ALA vs. placebo

Healthy population

A single trial from 1965 compared linseed oil (ALA 5.2 g/d) to sunflower seed oil (ALA 0.13 g/d) in 12,716 healthy adults. After 1 year of followup, no effect of ALA was found on risk of MI (OR=0.99; 95% CI 0.67, 1.45).[137]

At risk for CVD population

The same trial from 1965 compared linseed oil (ALA 5.2 g/d) to sunflower seed oil (ALA 0.13 g/d) in 452 adults with previous angina pectoris but no infarction. After 1 year of followup, those on ALA supplementation had a significantly lower risk of MI (OR=0.17; 95% CI 0.04, 0.79).[137]

CVD population

One ALA trial from the 1960s reported analyses of the effect of ALA in participants with a history of MI in a total of 438 people. The trial used linseed oil as the source of ALA (5.2 g/d) compared with sunflower seed oil (0.13 g/d ALA). It found no significant effect of ALA on risk of a subsequent MI (OR 0.84).[137]

RCT subgroup analyses

One trial of EPA+DHA 0.6 g/d versus placebo, in 2501 people with a history of any CVD found no difference in effect on risk of MI of marine oil in participants also taking B vitamins or not.[88]

Meta-regression of the marine oil trials found no significant interaction between n-3 FA dose (P=0.34), followup time (P=0.12), or between at risk and CVD populations (P=0.92)

Observational Studies

Three studies evaluated the associations between n-3 FA intake or biomarker level and MI risk in healthy adults, mostly men, in 4 to 11. 5 years (**Appendix Table M.3, Figure M.4**).[49, 95, 104, 133] Most analyses found no association. The Physicians Health Study found no association between intake of total n-3 FA (combined) and risk of MI in healthy men at 4 years of followup (plot #55). The two studies that evaluated marine oil (EPA+DHA) intake had different findings (plot #54). The Health Professional Follow-up Study found no significant association among healthy men at 6 years of followup.[49] The Japan Public Health Center-Based Study - Cohort I study found lower risk of MI among healthy adults (men and women combined) with higher EPA+DHA intake.[104]

Only the Physicians Health Study evaluated associations of n-3 FA biomarkers and MI. The study found no associations with cholesteryl ester or phospholipid levels of EPA, DHA, or combined EPA+DHA.

Table M.1. Myocardial infarction: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs Placebo											
Yokoyama 2007 17398308 Japan	At risk	EPA+Statin	EPA 1.8 g/d (Marine oil)	Statin	0	5 y	Local physicians monitored but compliance level was not reported	40/7503, 0.7%	51/7478, 0.5%	HR 0.79 (0.52, 1.19)	0.253
	CVD	EPA+Statin	EPA 1.8 g/d (Marine oil)	Statin	0	5 y	Local physicians monitored but compliance level was not reported	31/1823, 2.3%	42/1841, 1.7%	HR 0.75 (0.47, 1.19)	0.223
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	≤0.85 g (Marine oil) [E:D 0.9-1.5]	Placebo	0 (Olive oil)	5 y	Monitored by self-report but compliance level was not reported	10/6239, 0.2%	13/6266, 0.2%	HR 0.76 (0.34, 1.74)	0.52
Bosch 2012 22686415 Canada	CVD[d]	EPA+DHA	0.84 g/d (Marine oil) [E:D 1.24]	Placebo	0 (Olive oil)	6+ y	Followup (adherence was 88% at the end of study)	344/6281, 5.5%	316/6255, 5.1%	Adj HR 1.09 (0.93, 1.27)	0.28
Brouwer 2006 16772624 N Europe	CVD	EPA+DHA	0.96g n-3 PUFAs (0.464 g EPA, 0.335g DHA) (Marine oil) [E:D]=1.4	Placebo	0 (high-oleic acid sunflower oil)	1 y	Generally good (76% reported taking 80% pills) based on pill counts and confirmed by biomarkers.	1/273, 0.4%	3/273, 1%	OR 0.33 (0.03, 3.20)	0.339
Galan 2010 21115589 France	CVD	EPA+DHA	0.6 g/d (Marine oil) [E:D 2]	Placebo	0 (nd)	4.7 y	Patient reported (86% reported they took >=80% of allocated treatment)	51/1253, 4.1%	53/1248, 4.2%	Adj HR 0.97 (0.66, 1.42)	0.87
Sacks 1995 7759696 US	CVD	EPA+DHA+DPA	6 g/d (suppl) [E:D 1.5]	Placebo	0 (Olive oil)	2.4 y	Pill counts	1/31, 3.2%	2/28, 7.1%	OR 0.43 (0.04, 5.06)	0.505

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine oil) [E:D 0.83]	Placebo	0 (nd)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	107/3494, 3.1%	129/3481, 3.7%	Adj HR 0.82 (0.63, 1.06)	0.121
Burr 1989 2571009 UK	CVD	Fish advice, either alone or in combination with fiber advice, fat advice, or both fiber and fat advice.	EPA 0.34 g/d (diet)	No fish advice (Fat advice, fiber advice, fiber and fat advice, or no advice)	EPA 0.09 g/day (diet)	2 y	Compliance was good based on dietary assessments	127/1015, 12.5%	149/1018, 14.6%	Adj RR 0.84 (0.66, 1.07)	0.162
ALA vs Placebo											
Natvig 1965 5756076 Norway	Healthy	ALA	ALA 5.2 g/d (Linseed oil)	Control oil	ALA 0.13 g/d (Sunflower seed oil)	1 y	nd	52/6352, 0.8%	53/6364, 0.8%	OR 0.99 (0.67, 1.45)	NS
	At risk	ALA	ALA 5.2 g/d (Linseed oil)	Control oil	ALA 0.13 g/d (Sunflower seed oil)	1 y	nd	2/216, 0.9%	12/236, 5.1%	OR 0.17 (0.04, 0.79)	0.02
	CVD	ALA	ALA 5.2 g/d (Linseed oil)	Control oil	ALA 0.13 g/d (Sunflower seed oil)	1 y	nd	9/122, 7.4%	10/116, 8.6%	OR 0.84 (0.33, 2.16)	0.724

Figure M.2. Incident myocardial infarction: Randomized trials of marine oils

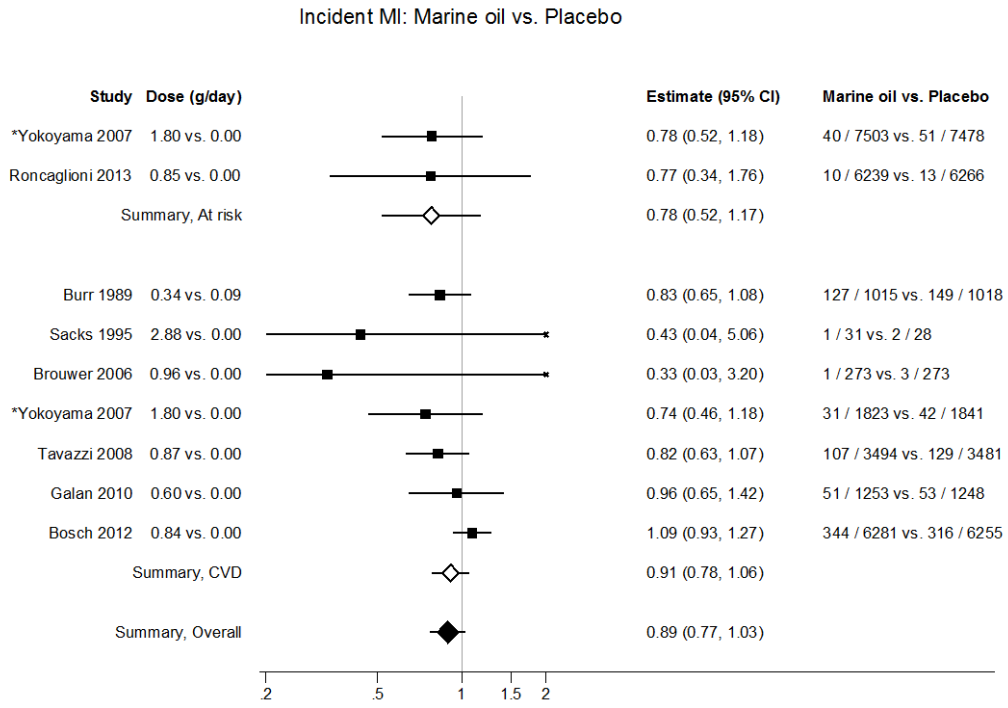
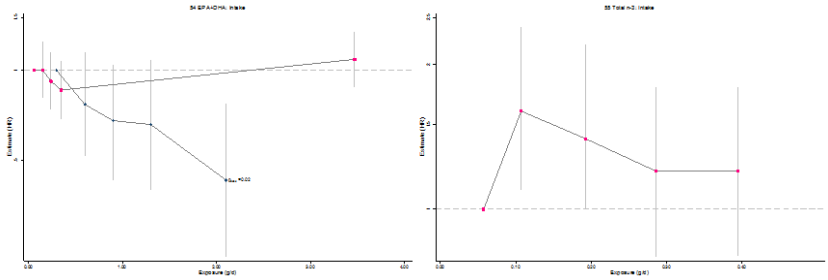


Figure M.4. n-3 FA associations with incident myocardial infarction: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for incident myocardial infarction. Studies that reported associations by continuous exposure (e.g., per g/day intake or per SD) are [currently] omitted. P values are the study-reported P value for the trend across quantiles.
Blue circles = healthy adults, pink squares = healthy males.

Revascularization

Randomized Controlled Trials

Five RCTs evaluated coronary revascularization as an outcome (**Table S.1**).[56, 88, 141, 150, 187] Of these, one was conducted in 18,645 hypercholesterolemic participants (19.5% with CAD),[187] and four were in a total of 20,469 participants with CVD including DM and history of CVD,[56] all CVD,[88] and MI[141, 150]

Marine oil vs. placebo

At risk for CVD population

Among 18,645 hypercholesterolemic participants (19.5% with CAD), one RCT compared 1.8 g/day EPA ethyl ester combined with statin with control (statin alone) for a duration of 5 years.[187] Adherence was not reported. There was no significant difference in the risk of coronary revascularization between the two groups (HR=0.86; 95% CI 0.71, 1.05).

CVD population

Among participants with CVD, four studies compared marine oil (EPA+DHA) to placebo (olive oil or corn oil).[56, 88, 141, 150] The 18,041 participants had a history of CVD or DM, CVD, or MI. The dose of EPA+DHA ranged from 0.6 to 1.7 g/day, and the EPA to DHA ratio ranged from 0.5 to 2. Reported in three studies, compliance was more than 70 percent. The mean duration of followup ranged from 1 to more than 6 years. All four studies found that EPA+DHA supplementation had no significant effect on revascularization compared with placebo with HR/OR ranging from 0.92 to 0.97.

Observational Studies

Only the Health Professional Follow-up Study analyzed coronary revascularization (**Appendix Table S.3, Figure S.4**).[49] The study found a nonsignificant association in healthy men between higher intake of combined EPA+DHA and *higher* risk of undergoing coronary artery bypass grafting after 6 years of followup (P=0.09).

Figure S.4. n-3 FA associations with coronary revascularization: Observational studies

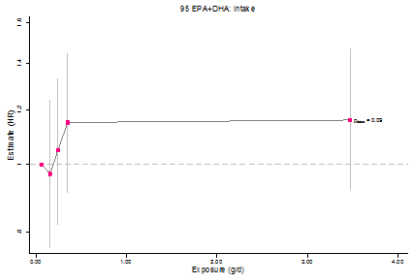


Table S.1. Revascularization: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	Outcome Definition	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs Placebo												
Yokoyama 2007 17398308 Japan	At risk	EPA	1.8 g/d (Marine oil)	Placebo	0	Coronary revascularization	5 y	nd	191/9326, 2%	222/9319, 2%	HR 0.86 (0.71, 1.05)	
Rauch 2010 21060071 Germany	CVD	EPA+DHA	0.46g EPA, 0.38g DHA (Marine oil) [E:D=1.2]	Placebo	0 (Olive oil)	Coronary revascularization	1 y	Pill counts at 3 mo and 12 mo (≥70% of study period)	~530/1919, 28%	~541/1885, 29%	OR 0.93 (0.80, 1.08)	
Bosch 2012 22686415 Canada	CVD[d]	EPA+DHA	0.84 g/d (Marine oil) [E:D 1.24]	Placebo	0 (Olive oil)	Coronary, carotid, aortic, or peripheral revascularization	6+ y	nd	866/6281, 14%	869/6155, 14%	HR 0.96 (0.87, 1.05)	0.39
Galan 2010 21115589 France	CVD	EPA+DHA	0.6 g/d (Marine oil) [E:D 2]	Placebo	0 (nd)	Coronary or peripheral revascularization	4.7 y	Patient reported (86% reported they took >=80% of allocated treatment)	152/1253, 12%	156/1248, 13%	HR 0.97 (0.78, 1.22)	0.82
Nilsen 2001 11451717 Scandinavia	CVD	EPA+DHA	1.7-1.764 g/d (Marine oil) [E:D 0.5]	Placebo	0 (Corn oil)	Coronary (implied) revascularization	2.4 y	82% in fish oil group; 86% in the placebo group	54/150, 36%	57/150, 39%	OR 0.92 (0.57, 1.47)	

Acute Coronary Syndrome

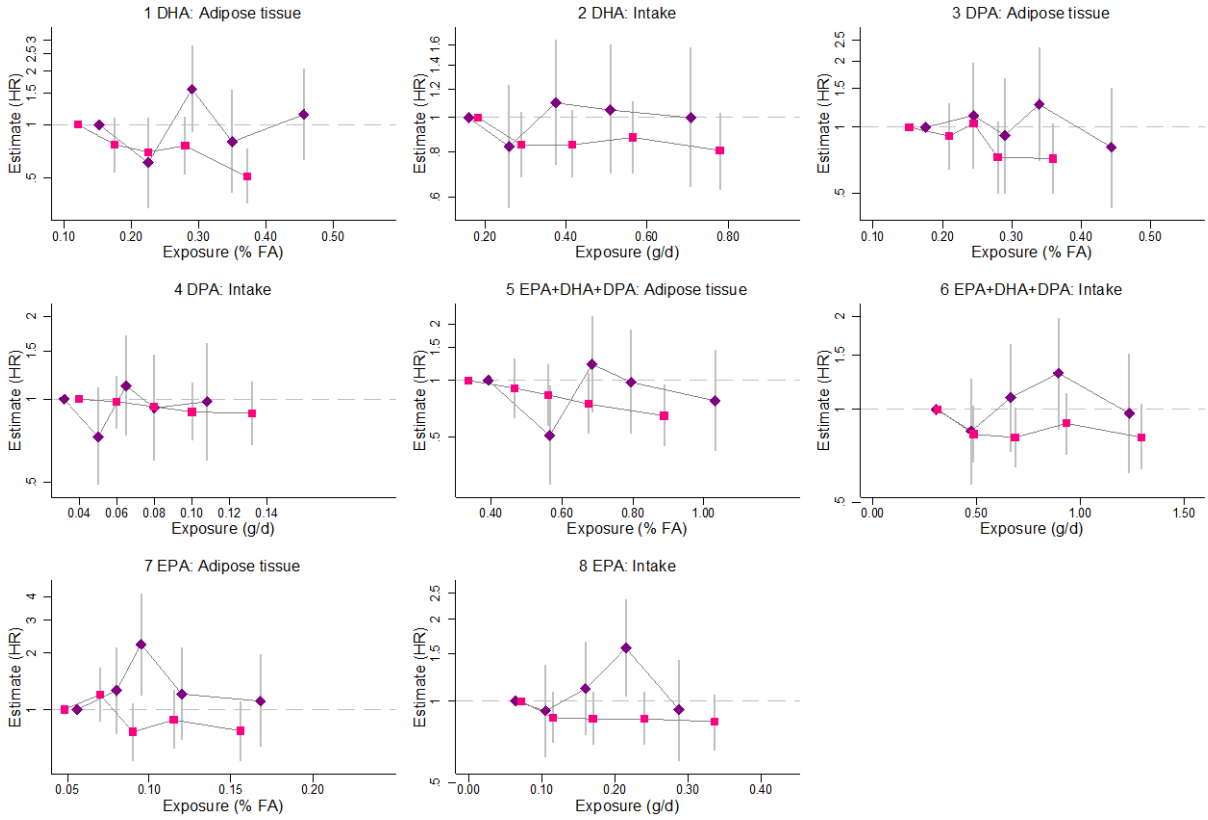
Randomized Controlled Trials

No RCT evaluated acute coronary syndrome (ACS).

Observational Studies

One study (Diet, Cancer, Health) evaluated the associations between multiple n-3 FA measures and acute coronary syndrome (MI or unstable angina) after a mean of 7.6 years in a healthy population (age 50-64 y) (**Appendix Table N.3, Figure N.4**).[108] Analyses were conducted separately for men and women; for DHA, DPA, EPA, and EPA+DHA+DPA; and for each n-3 FA type, both intake and adipose tissue percent FA. For both men and women, the intake levels of total n-3 FA were not associated with future acute coronary ischemia (plots #2, 4, 6, & 8). Among men, higher baseline adipose tissue DHA, DPA, and EPA+DHA+DPA, but not EPA, were significantly associated with decreased risk of acute coronary ischemia, based on both a 0.1 percent increase in baseline measure and comparing the highest and lowest quantiles for each n-3 FA adipose tissue level (plots #1, 3, 5, & 7). Among women, no statistically significant associations between baseline biomarker level and outcome were found.

Figure N.4. n-3 FA associations with acute coronary syndrome: Observational studies



Study (or cohort) level associations between n-3 FA exposure and hazard ratio (HR) for the outcome.
Pink squares = healthy males, purple diamonds = healthy females.

Angina Pectoris

Randomized Controlled Trials

Four trials evaluated angina pectoris (**Table P.1**). One RCT evaluated stable angina,[137] and three evaluated unstable angina.[141, 155, 187]

Marine oil vs. placebo

At risk for CVD population

One study compared 1.8 g/day purified EPA combined with statin with control (statin alone) to placebo with statin in 18,645 participants with dyslipidemia (19.5% with CAD).[187] Adherence was verified by local physicians at every clinic visit but the level was not reported. After 5 years of followup, the trial found a significant risk reduction in unstable angina pectoris events (HR 0.76 95% CI 0.62, 0.95) in participants who were assigned to the EPA+statin group compared to those in the statin alone group.

CVD population

Two trials were conducted in patients with documented CHD or MI.[141, 155] Among 359 patients followed for about 2.5 years, adherence was not reported but was verified through either pill counting or by local physicians who monitored compliance at every clinic visit. In one trial, EPA+DHA dose was 3.52 g/d (EPA to DHA ratio = 2); in the other total EPA+DHA+DPA dose was 6 g/d (EPA to DHA ratio = 1.5). Neither of the two studies reported a significant effect of marine oil on unstable angina pectoris compared with placebo (OR 0.64, 95% CI 0.13, 3.16; OR 1.18, 95% CI 0.67, 2.08).

ALA vs. placebo

Healthy population

One trial compared linseed oil (5.2 g/d ALA) to a control oil (sunflower seed oil, 0.13 g/d ALA) for a duration of 1 year among 13,628 generally healthy participants.[137] Adherence was verified at follow-up by participating physicians but level was not reported. This study found no significant effect on stable angina between the two groups (OR 1.58 95% CI 0.77, 3.26).

Observational Studies

No observational studies evaluated angina pectoris, per se.

Table P.1. Angina pectoris: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	Outcome Definition	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo												
Yokoyama 2007 17398308	At risk (dyslipidemia)	EPA+Statin	EPA 1.8 g/d (Marine oil)	Statin	0	Unstable Angina	5 y	Local physicians monitored compliance at every clinic visit (nd)	147 / 9326, 1.6%	193 / 9319, 2.1%	HR 0.76 (0.62, 0.95)	nd
Nilsen 2001 11451717	CVD	EPA+DHA	3.52 g/d (marine oil) [E:D 2]	Placebo	0 (Corn oil)	Unstable Angina	2.5 y	nd	32 / 150, 21.3%	28 / 150, 18.7&	OR 1.18 (0.67, 2.08)	0.564
Sacks 1995 7759696	CVD	EPA+DHA+DPA	6 g/d (suppl) [E:D 1.5]	Placebo	0 (Olive oil, potassium tablets)	Unstable Angina	2.4 y	Mean compliance determined by pill count	3 / 31, 9.6%	4 / 28, 14.3%	OR 0.64 (0.13, 3.16)	0.59
ALA vs. Placebo												
Natvig 1968 5756076	Healthy	ALA	ALA 5.2 g/d (Linseed oil)	Control oil	ALA 0.13 g/d (Sunflower seed oil)	Stable Angina	1 y	Participating physicians assessed compliance at follow-up (nd)	19 / 6641, 0.29%	12 / 6627, 0.18%	OR 1.58 (0.77, 3.26)	0.214

Stroke, Total (Ischemic and Hemorrhagic, Fatal and Nonfatal)

Randomized Controlled Trials

Seven RCTs evaluated total stroke (**Table Q.1**).[56, 88, 123, 126, 137, 155, 168] One was conducted in 13,406 healthy participants[137] and the other six included a total of 33,981 participants with CVD and/or DM,[56, 88] MI,[126, 155] persistent AFib,[123] and heart failure.[168]

Marine oil vs. placebo

CVD population

Six RCTs of participants with a history of CVD evaluated EPA+DHA supplementation.[56, 88, 123, 126, 155, 168] Followup duration ranged from 1 to at least 6 years. The total dose of marine oil ranged from 0.6 to 6 g/d. Among four of the studies the EPA to DHA ratio ranged from 0.8 to 2. None of the studies found a statistically significant effect of marine oil on risk of stroke, mostly with wide confidence intervals, and with effect sizes ranging from 0.92 (95% CI 0.79, 1.08) to 2.8 (95% CI 0.11, 71.6).

Across the six RCTs of CVD populations, the summary HR (**Figure Q.2**) was 1.02 (0.88, 1.19).

ALA vs. placebo

Healthy population

One RCT of 13,406 healthy participants compared linseed oil (5.2 g/d ALA) to a control oil (sunflower seed oil with 0.13 g/d ALA).[137] Adherence was not reported. After 1 year of follow up, the trial found no significant effect of ALA on stroke (OR=1.33; 95% CI 0.56, 3.16).

Observational Studies

Six studies evaluated the associations between n-3 FA intake or biomarker level and risk of total stroke in healthy adults in 4 to 16 years (**Appendix Table Q.3, Figure Q.4**).[70, 71, 85, 98, 105, 118, 133, 134] Most analyses found no association between n-3 FA intake and total stroke risk and all found no significant association with n-3 FA biomarker level.

n-3 FA Intake

All six studies evaluated n-3 FA intake (Cardiovascular Health Study, Health Professional Follow-up Study, MORGEN, Nurses' Health Study, Physician's Health Study, Swedish Mammography Study). Among analyses the only significant associations were found in women.

In a study of healthy men, the Physicians Health Study found no significant association between intake of total n-3 FA (combined) and total stroke after 4 years of followup (plot #78).

Three studies evaluated ALA intake (plot #72). Two studies (Cardiovascular Health Study, Swedish Mammography Study) found no significant association after 10 and 12 years. The third study, MORGEN, did not report a P value for trend across quintiles, but found lower risk for stroke in all quintiles (at 10 years) compared with the lowest, the middle three of which were statistically significant (with median intake above about 1.25 g/d).

Four studies evaluated combined EPA+DHA intake (plot #76). Three analyses were conducted in women and two in men. In analyses of women, MORGEN and the Swedish Mammography Study found significant associations between higher EPA+DHA (particularly with median intake of at least 0.56 g/d or >0.19 g/d) and lower risk of stroke at 10 years of followup, but the Nurses' Health Study found no significant association. Both the Health Professional Follow-up Study and the MORGEN analysis of men found no significant association.

n-3 FA Biomarkers

Two studies (Cardiovascular Health Study, MORGEN) evaluated plasma n-3 FA levels in healthy adults at 10 and 16 years. All analyses were not statistically significant. The Cardiovascular Health Study found no association with total n-3 FA plasma levels (combined, plot #79). Both studies found no association with plasma ALA (plot #73). In contrast with all other analyses, MORGEN found a nonsignificant *increased* risk of total stroke among adults with higher EPA+DHA levels measured as a continuous variable (P=0.07). The Cardiovascular Health Study found no associations for EPA, DHA, and DPA plasma levels (separately, plots #74, 75, & 77).

By meta-analysis (**Table Q.6**), overall there is no association between EPA+DHA±DPA intake and total stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 0.99 [95% CI 0.87, 1.12]). Meta-analyses with the addition of a spline knot point (from 0.1 to 0.5 g/d) found a best-fit curve with a change in slope (between g/d and risk of CHD) at 0.3 g/d, but both above and below this threshold the associations between intake and CHD were nonsignificant (<0.3 g/d: effect size per g/d = 0.62 [95% CI 0.35, 1.10]; >0.3 g/d: effect size per g/d = 1.09 [95% CI 0.94, 1.26]). Analyses at all thresholds between 0.1 and 0.5 g/d gave similar results.

Observational study subgroup analyses

The Cardiovascular Health Study found no difference in associations of ALA intake or plasma values and total stroke by amount of fish consumption at baseline or by sex. The Health Professional Follow-up Study found no difference in association between EPA+DHA intake and ischemic stroke based on whether participants used fish oil supplements.

Table Q.1. Stroke, Total: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs Placebo											
Bosch 2012 22686415 Canada	CVD[d]	EPA+DHA	EPA 0.465 g/d, DHA 0.375 g/d (marine oil) [E:D 1:1.24]	Placebo	0 (Olive oil)	≥6 y	Followup (adherence was 88% at the end of study)	314/6281, 5.0%	336/6255, 5.4%	HR 0.92 (0.79, 1.08)	0.32
Galan 2010 21115589 France	CVD	EPA+DHA	0.6 g/d (Marine oil) [E:D 2:1]	Placebo	0 (nd)	4.7 y	Patient reported (86% reported they took >=80% of allocated treatment)	29/1253, 2.3%	28/1248, 2.2%	HR 1.04 (0.62, 1.75)	0.88
Marchioli 2002 11997274 Italy	CVD	EPA+DHA	0.850-0.882 g/d (marine oil)	No intervention	nd	3.5 y	Followup (adherence was 72.5% at the end of study)	62/5666, 1.1%	57/5658, 1.0%	RR 1.22 (0.75, 1.97)	
Sacks 1995 7759696 U.S.	CVD	EPA+DHA+DPA	6 g/d (suppl) [E:D 1.5]	Placebo	0 (Olive oil)	2.4 y	Biomarker at followup	1/31, 3.2%	0/28, 0%	OR 2.8 (0.11, 71.63)	
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	0.850-0.882 g/d (marine oil) [E:D 1:1.2]	Placebo	0 (nd)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	122/3494, 3.5%	103/3481, 3.0%	HR 1.16 (0.89, 1.51)	0.271
Macchia 2013 23265344 Argentina and Italy	CVD	EPA+DHA	0.85-0.882 (suppl) [nd]	Placebo	0 (Olive oil)	1 y	nd	3/289, 1.0%	3/297, 1.0%	HR 1.16 (0.23, 5.78)	
ALA vs Placebo											
Natvig 1968 5756076 Scandinavia	Healthy	ALA	5.2 g/d (linseed oil)	Control oil	ALA 0.13 g/d (Sunflower seed oil)	1 y	nd	12/6716, 0.2%	9/6690, 0.1%	OR 1.33 (0.56, 3.16)	NS

Figure Q.2. Total stroke: Randomized trials of marine oils

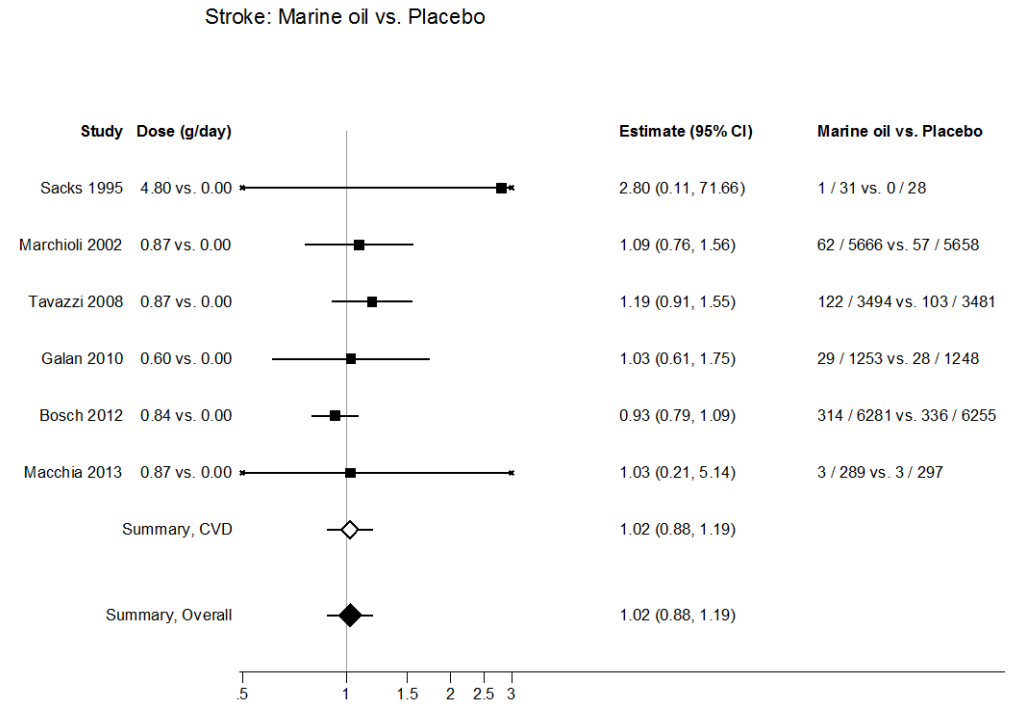


Figure Q.4. n-3 FA associations with total stroke: Observational studies

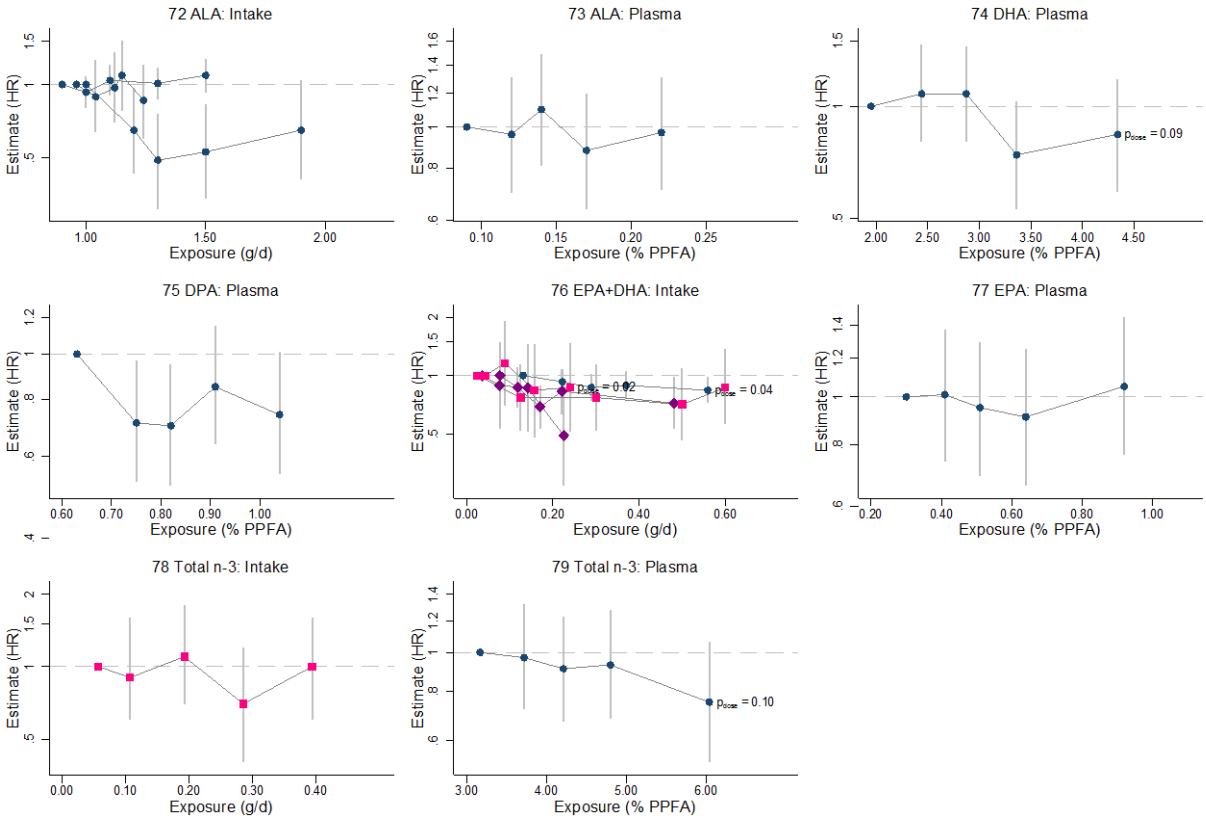


Table Q.6. Meta-analysis results of observational studies of marine oil (EPA+DHA±DPA) intake and total stroke

N Patients	Dose Range, g/d	Knot	Effect Size (ES), Overall	ES below knot	ES above knot	AIC	No. cohorts crossing threshold
178,249	0.025-0.60		0.99 (0.87, 1.12)			−1.8	5
		0.1		0.29 (0.07, 1.27)	1.06 (0.95, 1.19)	25.3	5
		0.2		0.52 (0.24, 1.13)	1.07 (0.95, 1.21)	20.2	5
		0.3		0.62 (0.35, 1.10)	1.09 (0.94, 1.26)	18.5	5
		0.4		0.68 (0.43, 1.06)	1.10 (0.94, 1.30)	19.1	5
		0.5		0.71 (0.50, 1.01)	1.12 (0.93, 1.34)	26.8	4

Stroke, Ischemic (Fatal and Nonfatal)

Randomized Controlled Trials

No RCTs evaluated ischemic stroke specifically.

Observational Studies

Five studies evaluated the associations between n-3 FA intake or biomarker level and risk of ischemic stroke in healthy adults after 10 to 22 years of followup (**Appendix Table R.3, Figure R.4**).[69, 71, 85, 93, 98, 105, 134, 184] All but one analysis across studies were nonsignificant for an association. All but two analyses across studies were nonsignificant for an association.

n-3 FA Intake

The five studies all evaluated n-3 FA intake (Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, Health Professional Follow-up Study, MORGEN, Nurses' Health Study). All found no association with ischemic stroke. This included two studies of ALA intake (Cardiovascular Health Study, MORGEN) in healthy adults after 10 and 12 years of followup (plot #63), one study of EPA and DHA intake, separately, measured as continuous variables (Atherosclerosis Risk in Communities Study) in healthy adults at 18 years followup, and four studies of combined EPA+DHA intake (plot #68) in one analysis of all healthy adults (Atherosclerosis Risk in Communities Study), two analyses in men (Health Professionals Follow-up Study, MORGEN), and two analyses in women (Nurses' Health Study, MORGEN).

By meta-analysis (**Table R.6**), overall there is a just-significant association between EPA+DHA±DPA intake and *higher* risk of ischemic stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 1.03 [95% CI 1.00, 1.07]). Meta-analyses with the addition of a spline knot point (from 0.1 to 0.5 g/d) found a best-fit curve with a change in slope (between g/d and risk of CHD) at 0.3 g/d, but both above and below this threshold the associations between intake and CHD were nonsignificant (<0.3 g/d: effect size per g/d = 0.77 [95% CI 0.27, 2.16], lower risk with increasing intake; >0.3 g/d: effect size per g/d = 1.06 [95% CI 0.86, 1.31], no or higher risk with increasing intake). Analyses at all thresholds between 0.1 and 0.5 g/d gave similar results.

n-3 FA Biomarkers

Three studies evaluated the association between n-3 FA biomarkers and risk of ischemic stroke (Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, MORGEN) in healthy adults.

The Cardiovascular Health Study found a significant association between plasma levels of total n-3 FA (combined) and lower risk of ischemic stroke in healthy adults ≥65 years of age after 16 years of followup (plot #71).

All three studies found no significant associations between plasma, cholesteryl ester, or phospholipid ALA levels and risk of ischemic stroke in healthy adults after 10, 16, and 22 years of followup (plot #64).

The Atherosclerosis Risk in Communities Study and the Cardiovascular Health Study found no associations between plasma, cholesteryl ester, or phospholipid EPA levels and risk of ischemic stroke in healthy adults after 16 to 22 years of follow-up (plot #70).

The same two studies evaluated DHA biomarkers (plot #65). The Atherosclerosis Risk in Communities Study found that those in the in the highest quintiles of DHA cholesteryl ester and phospholipid levels (separately) had lower risk of ischemic stroke with near-statistical significance (P=0.07 and 0.08, respectively). The Cardiovascular Health Study also found the same association across quintiles with plasma DHA with near statistical significance (P=0.052).

The Cardiovascular Health study also evaluated plasma DPA levels and found no significant association with ischemic stroke (plot #66).

The Atherosclerosis Risk in Communities Study found no significant association with cholesteryl ester or phospholipid EPA+DHA+DPA and ischemic stroke at 22 years of followup (plots #67 & 69), and also with phospholipid EPA+DHA at 18 years. MORGEN, however, found a statistically significant association between higher plasma EPA+DHA and ischemic stroke after 10 years.

Observational study subgroup analyses

The Cardiovascular Health Study found no difference in associations of ALA intake or plasma values and ischemic stroke by amount of fish consumption at baseline or by sex.

Figure R.4. n-3 FA associations with ischemic stroke: Observational studies

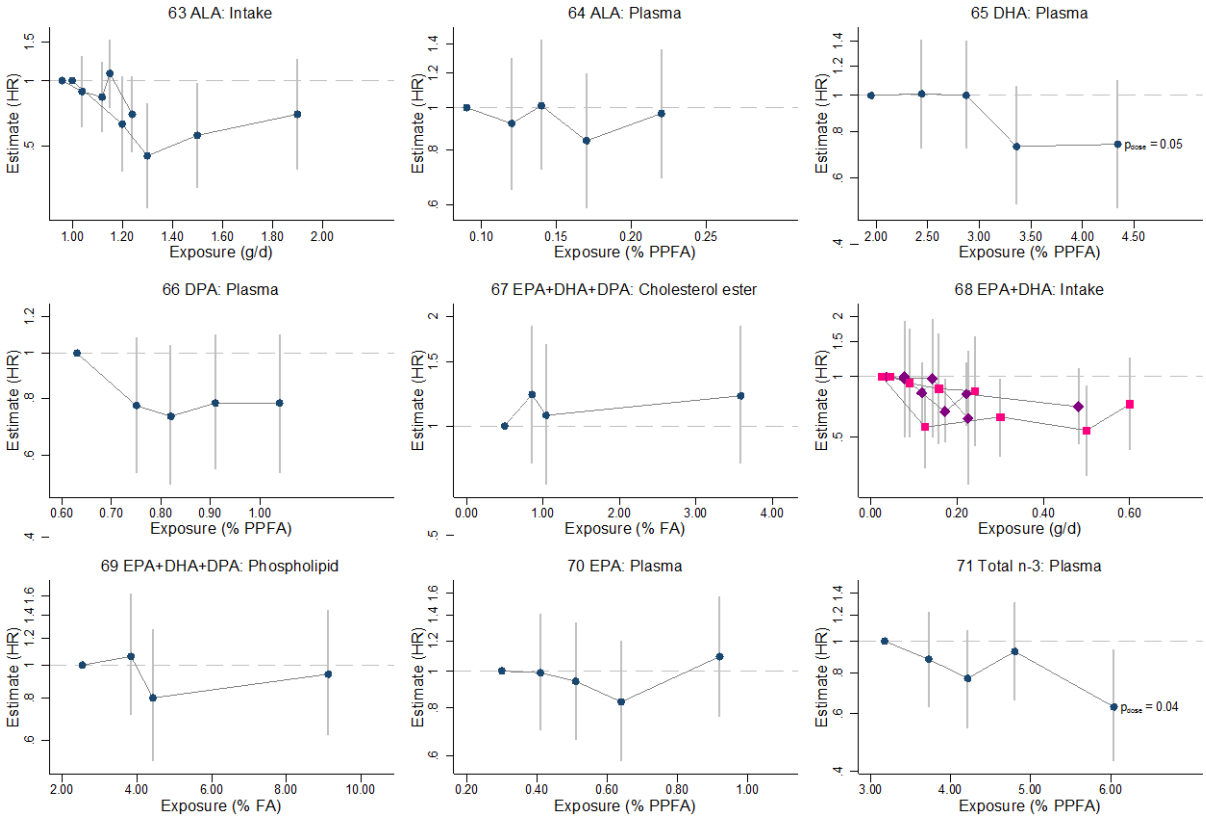


Table R.6. Meta-analysis results of observational studies of marine oil (EPA+DHA±DPA) intake and ischemic stroke

N Patients	Dose Range, g/d	Knot	Effect Size (ES), Overall	ES below knot	ES above knot	AIC	No. cohorts crossing threshold
143,579	0.025-0.60		1.03 (1.00, 1.07)			−4.4	4
		0.1		0.52 (0.17, 1.61)	1.04 (1.00, 1.09)	20.6	4
		0.2		0.68 (0.17, 2.75)	1.05 (0.88, 1.26)	17.2	4
		0.3		0.77 (0.27, 2.16)	1.06 (0.86, 1.31)	16.2	4
		0.4		0.83 (0.36, 1.89)	1.06 (0.82, 1.36)	16.6	4
		0.5		0.83 (0.59, 1.16)	1.07 (1.01, 1.13)	18.9	3

Stroke, Hemorrhagic (Fatal and Nonfatal)

Randomized Controlled Trials

No RCTs evaluated hemorrhagic stroke specifically.

Observational Studies

Five studies evaluated the associations between n-3 FA intake or biomarker levels and risk of hemorrhagic stroke in healthy adults after 10 to 16 years of followup (**Appendix Table T.3, Figure T.4**).[70, 71, 85, 98, 105, 120, 134] All but one analysis across studies were nonsignificant for an association.

n-3 FA Intake

The five studies all evaluated n-3 FA intake (Cardiovascular Health Study, Health Professional Follow-up Study, MORGEN, Nurses' Health Study, Swedish Mammography Study).

The Cardiovascular Health Study (in adults ≥65 years) and the Swedish Mammography Study in women both found no association between ALA intake and risk of hemorrhagic stroke (plot #56).

Four studies (Health Professional Follow-up Study, MORGEN, Nurses' Health Study, Swedish Mammography Study) evaluated EPA+DHA intake (plot #60). Only MORGEN, in a subgroup of men (<65 years old), found an association between higher EPA+DHA intake and *lower* risk of hemorrhagic stroke. No such association was found in women in MORGEN or the other three studies.

By meta-analysis (**Table T.6**), overall there is no association between EPA+DHA±DPA intake and risk of hemorrhagic stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 0.99 [95% CI 0.87, 1.12]). Meta-analyses with the addition of a spline knot point (from 0.1 to 0.5 g/d) found a best-fit curve with a change in slope (between g/d and risk of CHD) at 0.3 g/d, but both above and below this threshold the associations between intake and CHD were nonsignificant (<0.3 g/d: effect size per g/d = 0.62 [95% CI 0.35, 1.10], lower risk with increasing intake; >0.3 g/d: effect size per g/d = 1.09 [95% CI 0.94, 1.26], no or higher risk with increasing intake). Analyses at all thresholds between 0.1 and 0.5 g/d gave similar results.

n-3 FA Biomarkers

The Cardiovascular Health Study and MORGEN evaluated plasma n-3 FA and risk of hemorrhagic stroke. Both analyses found no significant associations, including total n-3 FA (combined, Cardiovascular Health Study, plot #62); ALA (both studies, plot #57); EPA (plot #61), DHA (plot #58), and DPA (plot #59) (Cardiovascular Health Study); and EPA+DHA (MORGEN).

Observational study subgroup analyses

The Cardiovascular Health Study found no difference in associations of ALA intake or plasma values and hemorrhagic stroke by amount of fish consumption at baseline or by sex.

Figure T.4. n-3 FA associations with hemorrhagic stroke: Observational studies

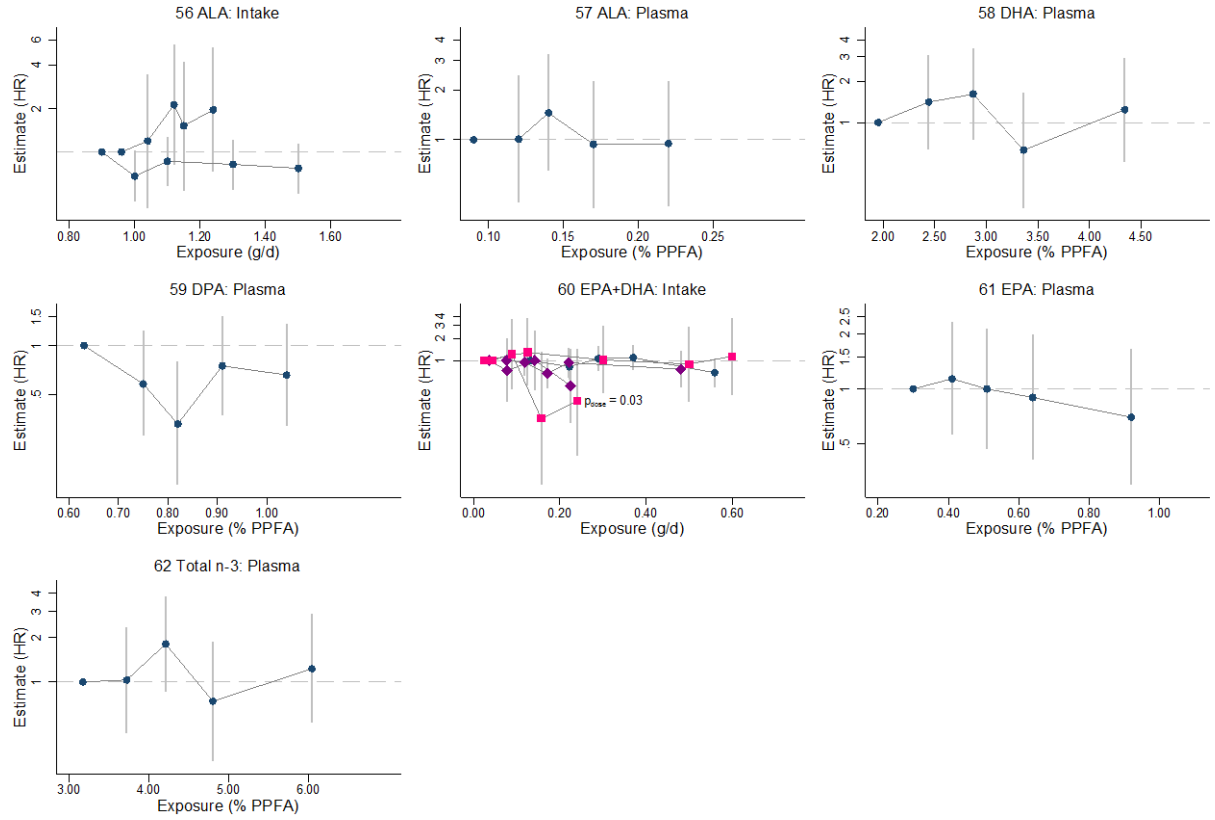


Table T.6. Meta-analysis results of observational studies of marine oil (EPA+DHA±DPA) intake and hemorrhagic stroke

N Patients	Dose Range, g/d	Knot	Effect Size (ES), Overall	ES below knot	ES above knot	AIC	No. cohorts crossing threshold
178,249	0.025-0.60		0.99 (0.87, 1.12)			−1.8	4
		0.1		0.29 (0.07, 1.27)	1.06 (0.95, 1.19)	25.3	5
		0.2		0.52 (0.24, 1.13)	1.07 (0.95, 1.21)	20.2	5
		0.3		0.62 (0.35, 1.10)	1.09 (0.94, 1.26)	18.4	5
		0.4		0.68 (0.43, 1.06)	1.10 (0.94, 1.30)	19.1	5
		0.5		0.71 (0.50, 1.01)	1.12 (0.93, 1.34)	26.8	4

Sudden Cardiac Death

Randomized Controlled Trials

Eight RCTs evaluated sudden cardiac death (SCD) (**Table U.1**).[56, 59, 116, 148, 150, 153, 168, 187] Of these, two studies were conducted in 27,486 participants at risk of CVD (defined as dyslipidemia[187] or with multiple risk factors[153]), and six in a total of 24,463 participants with CVD including DM and history of CVD,[56] arrhythmia,[59, 116, 148] MI,[150] and heart failure.[168]

Marine oil vs. placebo

Meta-analysis of the eight trials of marine oil yielded a nonsignificant summary HR=1.02 (95% CI 0.92, 1.14) (**Figure U.2**).[56, 59, 116, 148, 150, 153, 168, 187]

At risk for CVD population

Among people at risk for CVD, one study compared 1.8 g/day EPA ethyl ester combined with statin with control (statin alone) in 14,987 participants with dyslipidemia,[187] and one compared marine oil (EPA+DHA) or EPA to placebo in 12,505 participants with multiple risk factors.[153] The dose of EPA+DHA was at least 0.85 g/d. Neither study reported adherence level. The durations of followup was 4.6 and 5 years. Both studies found no significant differences in sudden cardiac death between groups (OR 1.24, 95% CI 0.36, 4.28; OR 1.28, 95% CI 0.88, 1.88).

Subgroup meta-analysis yielded a nonsignificant summary HR of 1.28 (95% CI 0.89, 1.84).

CVD population

Among people with existing CVD, five studies compared marine oil (EPA+DHA) to placebo (olive oil or oleic acid sunflower oil),[56, 59, 116, 148, 150, 168] Overall, these trials followed 24,463 people with existing CVD. The EPA+DHA doses ranged from 0.84 to 2.6 g/day. The duration of followup ranged from 0.8 years to 6.2 years. Compliance, when reported, ranged from 70 to 88 percent. All trials found no significant association, with effect sizes ranging from 0.94 to 3.06.

Across the four RCTs of CVD populations, the summary HR was 1.00 (0.90, 1.12).

RCT subgroup analyses

No trial reported a direct within-study subgroup analysis. By meta-regression of the marine oil trials, effect sizes did not vary across studies by dose (P=0.45), followup time (P=0.20), or population (P=0.42).

Observational Studies

Four studies evaluated the associations between multiple n-3 FA measures and SCD after about 11 to 18 years of follow-up in mostly healthy adults of varying ages, and also, in one study, women with a history of prior CVD (**Appendix Table U.3, Figure U.4**).[44, 45, 85, 104, 134] Analyses and studies found a mix of nonsignificant associations and associations favoring higher n-3 FA quantiles.

n-3 FA Intake

The Physician's Health Study found no association between total n-3 FA intake (combined) and risk of sudden cardiac death at 11 years in healthy men (plot #22). Two studies analyzed ALA intake (plots #15 & 16). The Nurses' Health Study found a significant association between higher ALA intake and lower risk of SCD after 18 years. The Cardiovascular Health Study found no significant association with 16 years of followup. The Japan Public Health Center-Based Study - Cohort I also found no association between EPA+DHA intake and risk of SCD at about 11.5 years (plot #20).

n-3 FA Biomarkers

The Cardiovascular Health Study found a significant association between plasma levels of total n-3 FA combined (implicitly ALA, DHA, DPA, and EPA) and lower risk of SCD with 16 years of followup (plot #23). The Cardiovascular Health Study, however, found no association between plasma ALA level and risk of SCD (plot #17). Regarding marine oils, this

same study found a significant association between plasma DHA (plot #18) and risk of SCD, but no significant associations with plasma DPA (plot #19) or EPA (plot #21).

Observational study subgroup analyses

In the Nurses' Health Study, the subgroup of women with no history of CVD at baseline had a significant association between higher ALA intake and lower risk of SCD after 18 years; in the smaller subgroup of women with a history of CVD, the effect estimates across quintiles were similar, but not statistically significant.[45] The Cardiovascular Health Study reported no significant difference (without details) in association between participants with high, low, or no fish consumption and between men and women.[85]

Table U.1. Sudden Cardiac Death: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs Placebo											
Yokoyama 2007 17398308 Japan	At risk	EPA (+Statin)	EPA 1.8 g/d (Marine oil)	Placebo (+Statin)	0	4.6 y	Monitored by local physicians	5/7503 0.0.07%	4/7478 0.05%	OR 1.24 (0.36 – 4.28)	0.743
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	>= 0.85 g/d (marine oil) [E:D 0.9:1-1.5:1]	Placebo	0 (Olive oil)	5 y	Self-reported (nd on level of adherence)	60/6239	47/6266	OR 1.28 (0.88, 1.89)	0.22
Bosch 2012 22686415 Canada	CVD[d]	EPA+DHA	EPA 0.465 g/d, DHA 0.375 g/d (Marine oil) [E:D 1.24]	Placebo	0 (Olive oil)	6.2 y	Followup (adherence was 88% at the end of study)	288/6281 4.6%	259/6255 4.1%	OR 1.11 (0.94, 1.32)	0.26
Leaf 2005 16267249 US	CVD	EPA+DHA	2.6 g/d (Marine oil)	Placebo	0 (Olive oil)	1 y	Pill counts and analysis of the phospholipids of red blood cells for their content of EPA and DHA. Noncompliance ~35%	3/200 1.5%	1/202 0.5%	3.06 (0.32, 29.68)	0.334
Rauch 2010 21060071 Germany	CVD	EPA+DHA	0.46g EPA, 0.38g DHA (Marine oil) [E:D 1.2]	Placebo	0 (Olive oil)	1 y	Pill counts at 3 months and 12 months (≥70% of study period)	28/1919 1.5%	29/1885 1.5%	0.95 (0.56, 1.6)	0.84
Raitt 2005 15956633 US	CVD	EPA+DHA	EPA 0.756 g/d, DHA 0.54 g/d (fish oil) [E:D 1.4]	Placebo	0 (olive oil: 73% oleic acid, 12%)	2 y	RBC and plasm Omega 3 FA levels	2.5/100.5 2.5%	0.5/100.5 0.5%	5.1 (0.24, 107.64)	0.47
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine oil) [E:D 0.83]	Placebo	0 (NR)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	307/3494	325/3481	OR 0.94 (0.79, 1.1)	0.333
Brouwer 2006 16772624 Europe	CVD	EPA+DHA	0.96g n-3 PUFAs (0.464 g EPA, 0.335g DHA) (Marine oil) [E:D=1.4]	Placebo	0 (high-oleic acid sunflower oil)	1 y	Generally good (76% reported taking 80% pills) based on pill counts and confirmed by biomarkers.	81/273 29.7%	90/273 33%	0.86 (0.6, 1.23)	0.33

Figure U.2. Sudden cardiac death: Randomized trials of marine oils

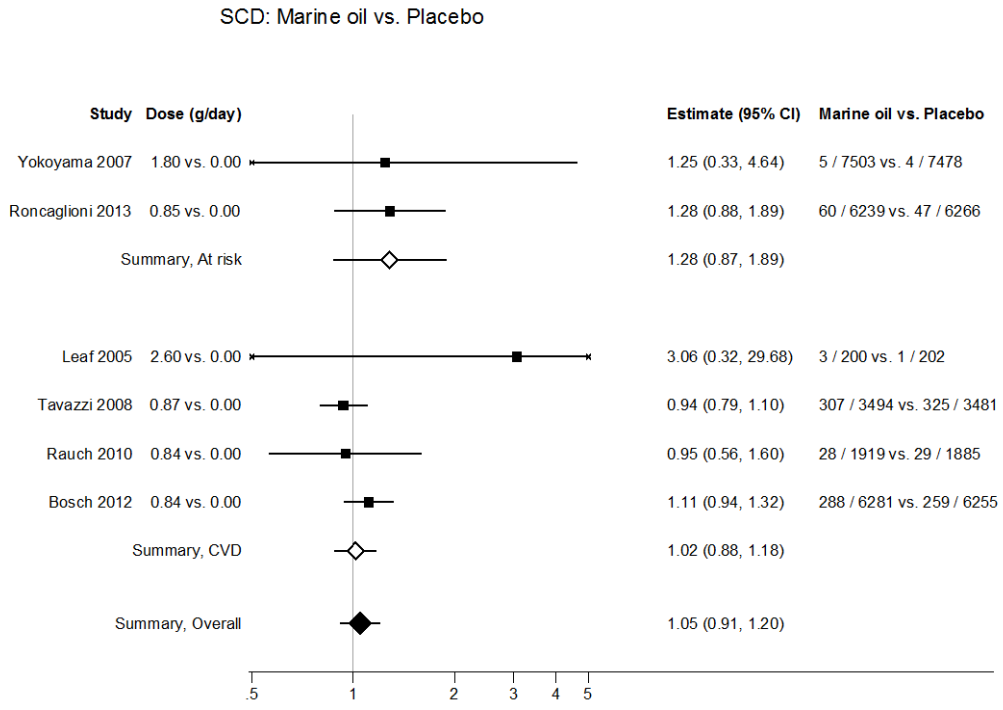
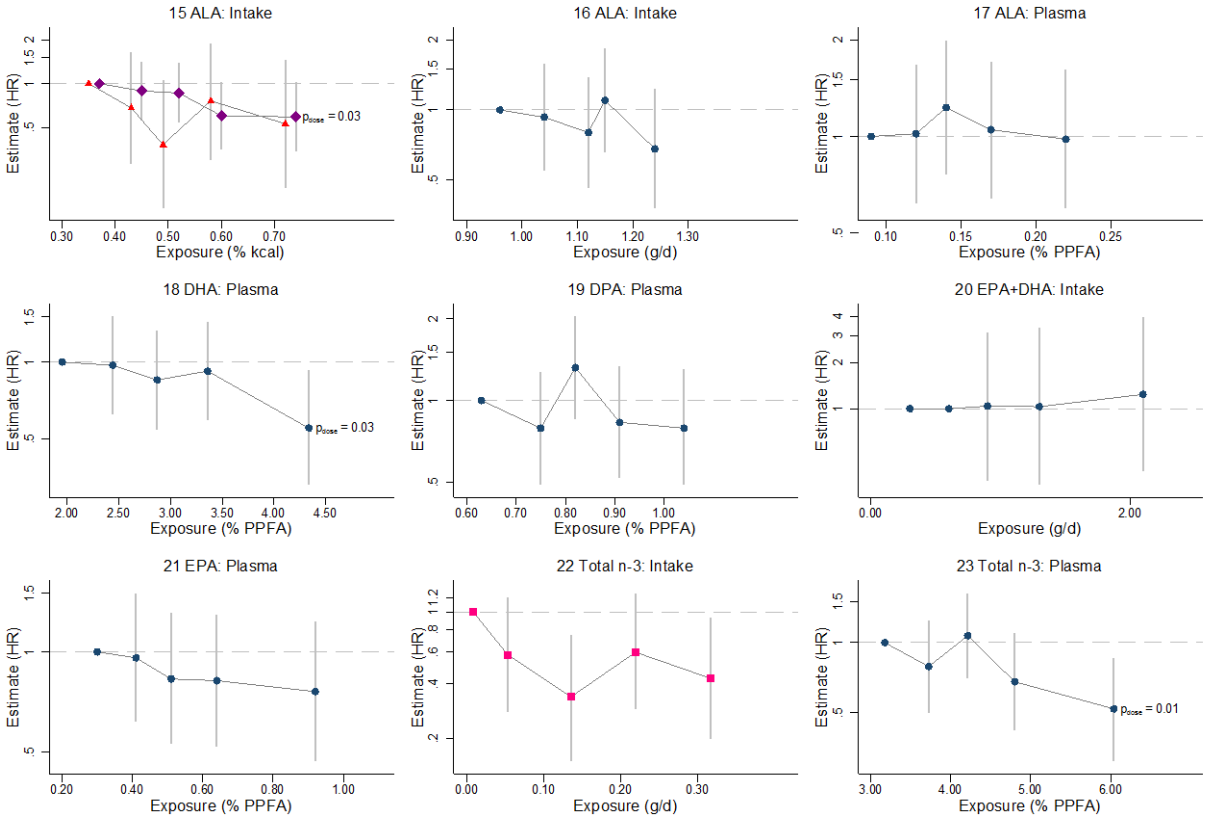


Figure U.4. n-3 FA associations with sudden cardiac death: Observational studies



Atrial Fibrillation

Randomized Controlled Trials

Three RCTs evaluated atrial fibrillation (AFib).[123, 142, 168] All were conducted among people with CVD (**Table V.1**). Specifically, two studies were conducted in a total of 785 people who had previous persistent AFib,[123, 142] and one in 5835 heart failure patients without AFib at study entry.[168]

Marine oil vs. placebo

CVD population

Among 785 people with previous persistent AFib, two RCTs compared marine oil (EPA+DHA) to placebo (olive oil).[123, 142] The same dose of EPA+DHA (0.850 to 0.882 g/d) was used in both studies for a duration of 1 year, but the EPA to DHA ratio was 0.5 in one study and 1.2 in another. Compliance was not reported. Both studies found that EPA+DHA supplementation had no significant effect on the recurrence of AFib (HR 1.28; 95% CI 0.90, 1.83; OR 0.52, 95% 0.26, 1.06).

Among 5835 heart failure patients without AFib at study entry, one RCT compared marine oil (EPA+DHA) to placebo (source not reported).[168] The dose of EPA+DHA was 0.850 to 0.882 g/d with a EPA to DHA ratio of 1.2. Compliance was about 70 percent. This study found no significant effect on incidence of AFib comparing EPA+DHA to placebo after a mean 3.9 years of followup (HR 1.10 95% CI 0.96, 1.25).[168]

RCT subgroup analyses

In one trial of AFib recurrence in people with a history of persistent AFib,[123] no differences in effect were found between subgroups based on sex, age (at a threshold of 60 years), or duration of prior AFib (at a threshold of 48 hours). In the trial of incident AFib (history of heart failure),[168] no differences in effect were found between subgroups based on age (threshold 70 years), left ventricular ejection fraction (threshold 40%), ischemic versus nonischemic heart failure, New York Heart Association class (I&II vs. III&IV), diabetes, total cholesterol (200 mg/dL threshold), glomerular filtration rate (60 mL/min threshold), or fish intake (2 servings per week threshold).

Observational Studies

Four studies evaluated the associations between multiple n-3 FA measures and AFib after 6.4 to 16 years of followup in healthy adults (mostly over age 50 or 65 years) (**Appendix Table V.3, Figure V.4**).[53, 58, 84, 86, 182] Most specific analyses found no significant association and the two studies with significant associations were inconsistent.

n-3 FA Intake

All four studies evaluated n-3 FA intake. The Cardiovascular Health Study found no significant association with ALA intake (plot #9), overall and, separately, in men and women. The other three studies (Women's Health Initiative, Rotterdam, and the Diet, Cancer, Health study) evaluated marine oil (EPA+DHA±DPA) intake (plot #13). Over a relatively low and narrow range of marine oil intake (less than about 0.3 g/d), the Women's Health Initiative and

Rotterdam studies found no significant association. In contrast, the Diet, Cancer, Health study found that higher EPA+DHA+DPA intake, particularly in the quintile with median intake of 1.3 g/d, was associated with lower risk of AFib in healthy women age 50 to 64 years was associated with higher risk of AFib or flutter after a mean of 8.1 years.

n-3 FA Biomarkers

Only the Cardiovascular Health Study evaluated biomarkers. The study found significantly lower risks of AFib (after 14 years) with higher plasma levels of total n-3 FA combined (not plotted because median quantile values not reported), and DHA (plot #11) in healthy adults at least 65 years of age. No significant associations were found with plasma ALA (plot #10), DPA (plot #12), or EPA (plot #14).

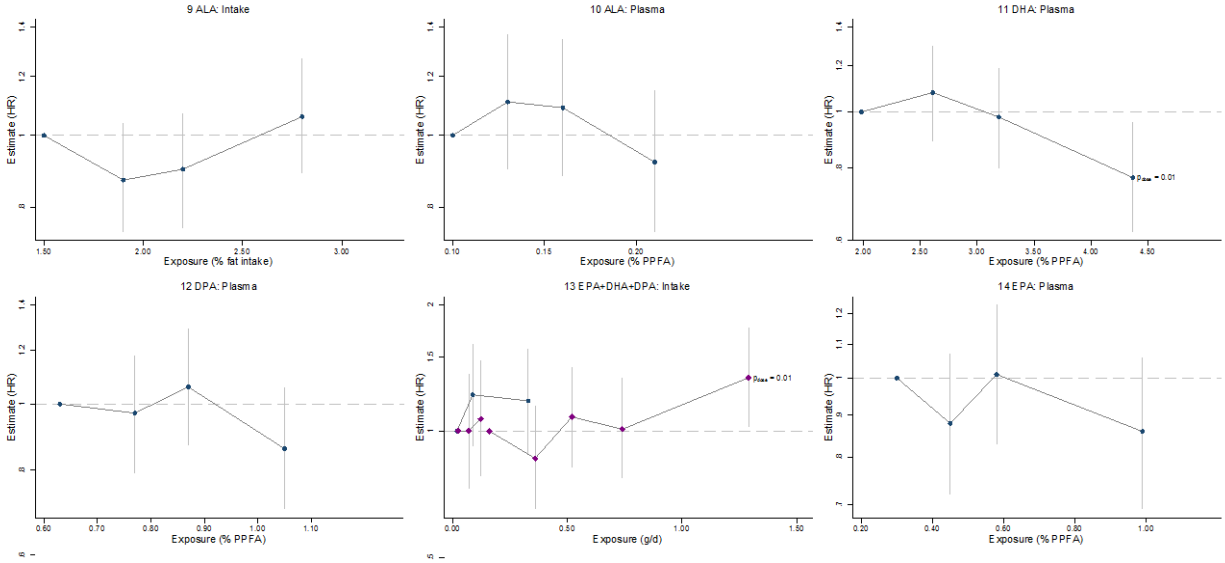
Observational study subgroup analyses

In the Cardiovascular Health Study, no differences were found (in a lack of association) for either plasma levels or intake of ALA and AFib between men and women.

Table V.1. Atrial Fibrillation: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N, %	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo											
Macchia 2013 23265344 Italy & Argentina	CVD (previous persistent Afib)	EPA+DHA	0.850-0.882 g/d (marine oil) [E:D 0.5]	Placebo	0 (Olive oil)	1 y	NR	56 / 297, 18.9%	69 / 289, 23.9%	HR 1.28 (0.90, 1.83)	0.17
Nodari 2011 21844082 Italy	CVD (previous persistent Afib)	EPA+DHA	0.850-0.882 g/d (marine oil) [E:D 1.2]	Placebo	0 (Olive oil)	1 y	NR	15 / 100, 15%	25 / 99, 25%	OR 0.52 (0.26, 1.06)[a]	NR
Tavazzi 2008 18757090 Italy	CVD (heart failure, no history of AFib)	EPA+DHA	0.850-0.882 g/d (Marine oil) [E:D 0.83]	Placebo	0 (NR)	3.9 y	Exam question (~30% not taking n-3 FA or placebo by the end of study)	444/2921, 15.2%	408/2914, 14.0%	HR 1.10 (0.96, 1.25)	0.11

Figure V.4. n-3 FA associations with atrial fibrillation: Observational studies



Congestive Heart Failure

Randomized Controlled Trials

Three RCTs evaluated congestive heart failure (CHF), all of which evaluated marine oils and had as an endpoint CHF hospitalization (**Table W.1**).[123, 153, 155]

Marine oil vs. placebo

At risk for CVD population

Among 12,505 people with multiple risk factors for CVD, one RCT compared marine oil (EPA+DHA) to placebo for a median duration of 5 years.[153] The dose of EPA and DHA was at least 0.85 g/d (composition of the marine oil was not reported). Adherence was verified by participants' self-report but the level of adherence was not reported. The trial found a significant risk reduction in CHF hospitalizations in participants who were assigned to marine oil group compared with those in placebo group (HR 0.67; 95% CI 0.52, 0.87).

CVD population

Among people with CVD, two RCTs compared marine oil (EPA+DHA) to placebo.[123, 155] The two RCTs compared marine oil (EPA+DHA) to placebo included a total of 645 CVD patients. The dose of EPA and DHA ranged from 0.85 to 0.882 g/d in one study (adherence was not reported) and was 6 g/d in the other study (adherence was 80% for EPA+DHA+DPA; 90% for placebo). The duration of followup ranged from 1 to 2.4 years. These two studies found no significant effects on CHF hospitalizations comparing marine oil to placebo (0/31 vs. 1/28, P value not reported; HR 0.85; 95% CI 0.26, 2.81).

Observational Studies

Eight studies evaluated the associations between intake and biomarkers of n-3 FA and CH) (**Appendix Table W.3, Figure W.4**).[52, 58, 96, 117, 119, 120, 135, 180, 186] Definitions of CHF outcomes varied across studies, including incident CHF and CHF hospitalization. One study analyzed only people with a history of MI; the Cohort of Swedish Men also reported a subgroup analysis in people with either diabetes of a history of MI. The remaining analyses were conducted in generally healthy populations. The median followup duration across studies was 9.5 years (range of average followup 4 to 16 years). Studies found a mix of both significant associations between higher n-3 FA intake or biomarker levels and lower risk of CHF and lack of association.

n-3 FA Intake

Six studies evaluated n-3 FA intake and CHF (Cardiovascular Health Study, Cohort of Swedish Men, Physician's Health Study, Rotterdam, Swedish Mammography Study, Women's Health Initiative). All but one analysis found no associations between n-3 FA intake and CHF.

The four studies assessing ALA intake (Cardiovascular Health Study, Physician's Health Study, Swedish Mammography Study, Women's Health Initiative) found no association with incident CHF or CHF hospitalization or death across 4 to 12 years of followup of healthy adults (plot #46).

Among the five studies evaluating EPA+DHA or EPA+DHA+DPA intake (Cohort of Swedish Men, Physician's Health Study, Rotterdam, Swedish Mammography Study, Women's Health Initiative), only the Swedish Mammography Study found an association between higher marine oil intake at baseline and CHF (hospitalization or death) in healthy women after 9 years of followup (plot #51). The Cohort of Swedish Men study, in contrast found no association after 7 years of followup, including in a subgroup analysis of men with a history of MI or diabetes at baseline.

By meta-analysis (**Table W.6**), overall there is a near significant association between higher marine oil intake and decreased risk of CHF across a median dosage range of 0.014 to 0.71 g/d (effect size per g/d = 0.80 [95% CI 0.62, 1.03]). Meta-analyses with the addition of a spline knot point (from 0.1 to 0.6 g/d) found a best-fit curve with a change in slope (between g/d and risk of CHF) at 0.2 g/d, with a significant reduction in risk of CHF up to 0.2 g/d (effect size per g/d = 0.45 [95% CI 0.28, 0.72]), but no significant association (trending toward increased risk) above 0.2 g/d (effect size per g/d = 1.06 [95% CI 0.98, 1.16]). Analyses at thresholds between 0.1 and 0.5 g/d gave similar results.

n-3 FA Biomarkers

Four studies conducted numerous analyses of n-3 FA biomarkers (Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, Osaka Acute Coronary Insufficiency Study, Physician's Health Study) in healthy adults (3 studies) and adults with a history of MI (Osaka Acute Coronary Insufficiency Study) with 4 or 14 years of followup.

One study (Cardiovascular Health Study) found lower incidence of CHF in adults ≥65 years old after 14 years of followup with higher plasma levels of total n-3 FA combined, but the association was not quite statistically significant (P=0.062) (plot #49).

Three studies analyzed plasma, cholesteryl ester, and phospholipid ALA (Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, Physician's Health Study) (plots #47 & 48). Only the Physicians Health study found an association of lower risk of CHF in men with

higher plasma ALA levels after 4 years of followup; the Cardiovascular Health Study found no such association in adults ≥65 years at 14 years of followup and the Atherosclerosis Risk in Communities Study found no association with either cholesteryl ester or phospholipid ALA in younger adults (45-64 years old) also at 14 years of followup.

Three studies analyzed blood, plasma, cholesteryl ester, and phospholipid EPA (Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, Osaka Acute Coronary Insufficiency Study). The studies had heterogeneous findings. The Cardiovascular Health Study found that higher plasma EPA levels were associated with lower risk of CHF in older adults (>65 y) with 14 years of followup (plot #53) (in contrast to a lack of association for DHA [plot #49]). The Osaka Acute Coronary Insufficiency Study also found a significant association between higher blood EPA levels and lower risk of CHF in adults with a history of MI (4 year followup), also in contrast with their finding for DHA (no association). The third study, the Atherosclerosis Risk in Communities Study, found no significant associations with either cholesteryl ester phospholipid DHA and CHF, with no difference in associations between men and women. These findings were also in contrast to their finding for DHA.

The same three studies analyzed the same DHA biomarkers, with heterogeneous findings. The Cardiovascular Health Study found no association with plasma DHA in healthy older adults (≥65 years, 14 year followup) (plot #49), in contrast with an association found for plasma EPA. The Osaka Acute Coronary Insufficiency Study also found no association with blood DHA in adults with a history of MI (4 year followup), in contrast to an association found for EPA. found a significant difference in association between men and women for both cholesteryl ester and phospholipid DHA.

Only the Cardiovascular Health Study evaluated plasma DPA (plot #50), in healthy older adults (≥65 years) with 14 years of followup. CHF risk was lower in participants with higher plasma DPA levels with near statistical significance (P=0.057).

Two studies analyzed biomarkers for combined marine oils. The Physicians Health Study found no association between plasma EPA+DHA+DPA and CHF risk in healthy men at 4 years (plot #52). The Atherosclerosis Risk in Communities Study also found no association with cholesteryl ester or phospholipid EPA+DHA+DPA and CHF in healthy men after 14 years. In women, no association was found with cholesteryl ester EPA+DHA+DPA, but higher levels of phospholipid EPA+DHA+DPA were associated with lower CHF risk.

Observational study subgroup analyses

The Cardiovascular Health Study found no differences in associations between ALA plasma or intake levels and CHF in subgroups based on age, sex, diabetes, or fish consumption (**Table W.5**).[117]

The Osaka Acute Coronary Insufficiency Study conducted multiple subgroup analyses for the associations between blood DHA, blood EPA, and CHF.[96] For both biomarkers, no significant interaction between subgroups and associations were found for use of angiotensin receptor blocker drugs, use of beta blocker drugs, diabetes, dyslipidemia, hypertension, glomerular filtration function (threshold = 60 mL/min), or hypertriglyceridemia (threshold = 150 mg/dL). Statistically significant interactions were found for statin use. In participants taking statins, risk of CHF was not associated with blood DHA (HR=0.74) or EPA (HR=1.45) levels were not associated with risk of CHF, in contrast with significant associations among participants not taking statins: DHA HR=6.65 (P interaction = 0.003); EPA HR=6.40 (P interaction = 0.048). Similarly for baseline HDL-c level, a significant interaction was found for

blood EPA (P interaction = 0.034) and a near-significant interaction for blood DHA (P interaction = 0.096), such that significant associations were seen in participants with low HDL-c (<40 mg/dL), but not among those with higher HDL-c. Subgroup analyses by sex found a significant interaction (P interaction = 0.008) with blood EPA, but not blood DHA, such that in men there was a significant association between EPA and CHF risk (HR=3.48) but not among women (HR=0.88). Near-significant interactions were found for blood DHA and age (P interaction = 0.051, significant association found for those ≥65 years old) and LDL-c (P interaction = 0.068, significant association found for those with LDL-c <100 mg/dL) (Table W.5). No interactions were found for blood EPA.

The Cohort of Swedish Men found no differences in associations of EPA+DHA intake and CHF between men with histories of diabetes or MI and healthy men, or between those who used marine oil supplements or not.[119]

Table W.1. Congestive Heart Failure Hospitalization: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int n/N,%	Ctrl n/N,%	Effect Size	Reported P value
Marine oil vs. Placebo											
Roncaglioni 2013 23656645 Italy	At risk	EPA+ DHA	≥0.85 g/d (suppl) [E:D 0.9-1.5]	Placebo	0 (Olive oil)	5 y	Self-reported (nd on level of adherence)	96/6239, 5%	142/6266, 2.3%	HR 0.67 (0.52, 0.87)	0.002
Macchia 2013 23265344 Argentina; Italy	CVD	EPA+ DHA	0.85-0.882 g/d (suppl) [nd]	Placebo	0 (Olive oil)	1 y	nd	5/289, 1.7%	6/297, 2.0%	HR 0.86 (0.26, 2.81)	nd
Sacks 1995 7759696 US	CVD	EPA+ DHA+ DPA	6 g/d (suppl) [E:D 1.5]	Placebo	0 (Olive oil)	2.4 y	Pill counting (80% for EPA+DHA; 90% for placebo)	0/31, 0%	1/28, 3.6%	nd	nd

Table W.5. Congestive Heart Failure: Subgroup Analyses, Observational studies

Study	Subgroups	n-3 FA	N Total	P difference	Difference	Favors
Cardiovascular Health Study[117]	Fish consumption vs low or no fish consumption (<0.6 servings/week)	ALA (Plasma or Intake)	4432	NS		
	Men vs Women			NS		
	Age, continuous			NS		
	Diabetes vs. no diabetes			NS		
	Body mass index, continuous			NS		
	Plasma linoleic acid, continuous			NS		
Osaka Acute Coronary Insufficiency Study[96]	Age <65 vs ≥65 years	DHA (Blood)	671	0.051	0.52 vs. 3.00	≥65 y
	Male vs Female			0.37		
	Diabetes vs. no diabetes			0.61		
	Hypertension vs. no hypertension			0.13		
	Dyslipidemia vs. no dyslipidemia			0.15		
	LDL-c <100 vs ≥100 mg/dL			0.068	3.48 vs. 0.88	Low LDL-c
	HDL-c <40 vs ≥40 mg/dL			0.096	4.50 vs. 1.17	Low HDL-c
	Tg <150 vs. ≥ 150 mg/dL			0.66		
	eGFR <60 vs. ≥60 mL/min			0.27		
	Statin vs no statin			0.003	0.74 vs. 6.65	No statin
	ACEi/ARB vs. no ACEi/ARB			0.39		
	Beta blocker vs. no beta blocker			0.37		
	Age <65 vs ≥65 years	EPA (Blood)	671	0.44		
	Male vs Female			0.008	5.82 vs. 0.69	Male
	Diabetes vs. no diabetes			0.98		
	Hypertension vs. no hypertension			0.84		
	Dyslipidemia vs. no dyslipidemia			0.14		
	LDL-c <100 vs ≥100 mg/dL			0.68		
	HDL-c <40 vs ≥40 mg/dL			0.034	15.7 vs. 1.44	Low HDL-c
	Tg <150 vs. ≥ 150 mg/dL			0.97		
	eGFR <60 vs. ≥60 mL/min			0.94		
	Statin vs no statin			0.048	1.45 vs. 6.40	No statin
	ACEi/ARB vs. no ACEi/ARB			0.17		
	Beta blocker vs. no beta blocker			0.27		
Cohort of Swedish Men[119]	History of DM or MI vs. healthy	EPA+DHA (Intake)	5234	NS		
	Supplement use vs. no supplement			NS		

Figure W.4. n-3 FA associations with congestive heart failure: Observational studies

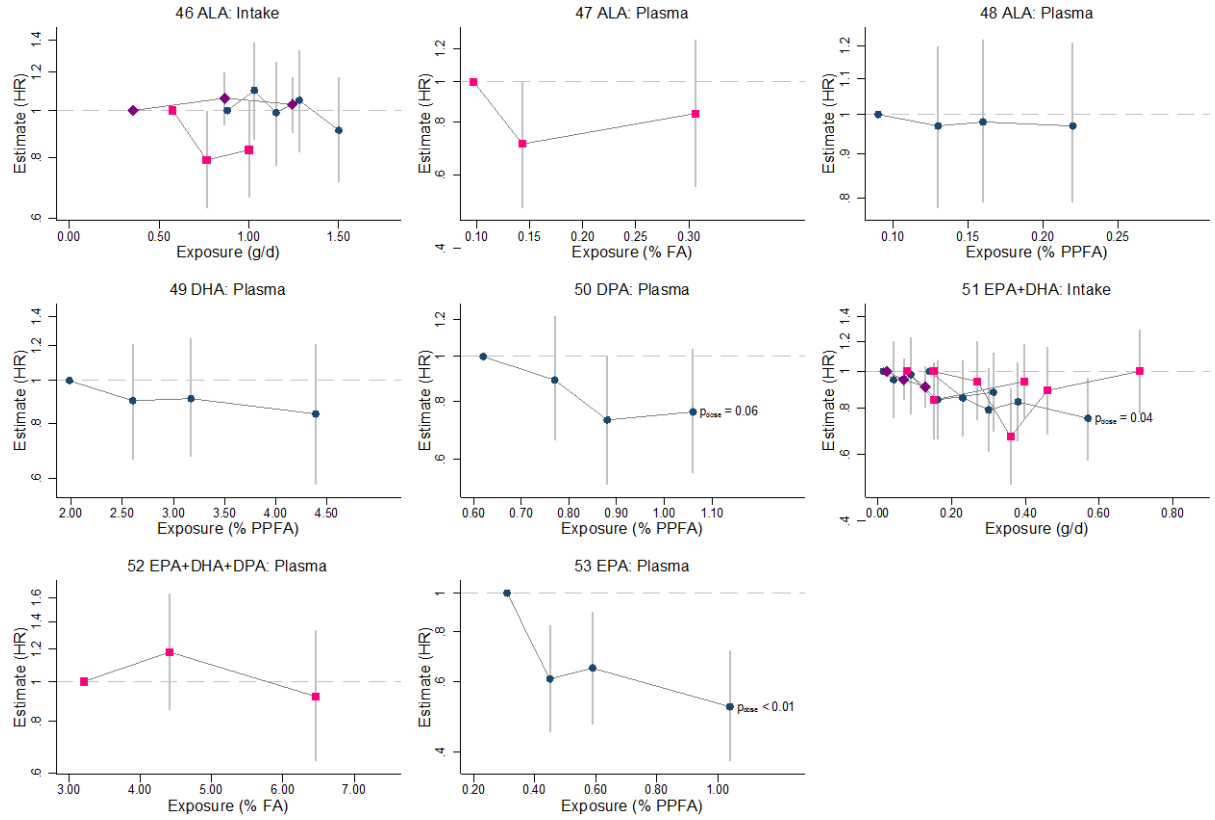


Table W.6. Meta-analysis results of observational studies of ALA intake and CHD

N Patients	Dose Range, g/d	Knot	Effect Size (ES), Overall	ES below knot	ES above knot	AIC	No. cohorts crossing threshold
184,491	0.014-0.71	NA	0.80 (0.62, 1.03)			−0.5	5
		0.10		0.22 (0.06, 0.77)	1.04 (0.72, 1.50)	24.2	5
		0.20		0.45 (0.28, 0.72)	1.06 (0.98, 1.16)	19.9	5
		0.30		0.57 (0.42, 0.79)	1.07 (0.98, 1.17)	23.4	5
		0.40		0.64 (0.48, 0.84)	1.17 (0.74, 1.84)	27.8	4
		0.50		0.67 (0.50, 0.88)	1.70 (0.67, 4.35)	32.9	4
		0.60		0.71 (0.55, 0.91)	4.64 (0.53, 40.99)	49.1	2

Hypertension, Incident

Randomized Controlled Trials

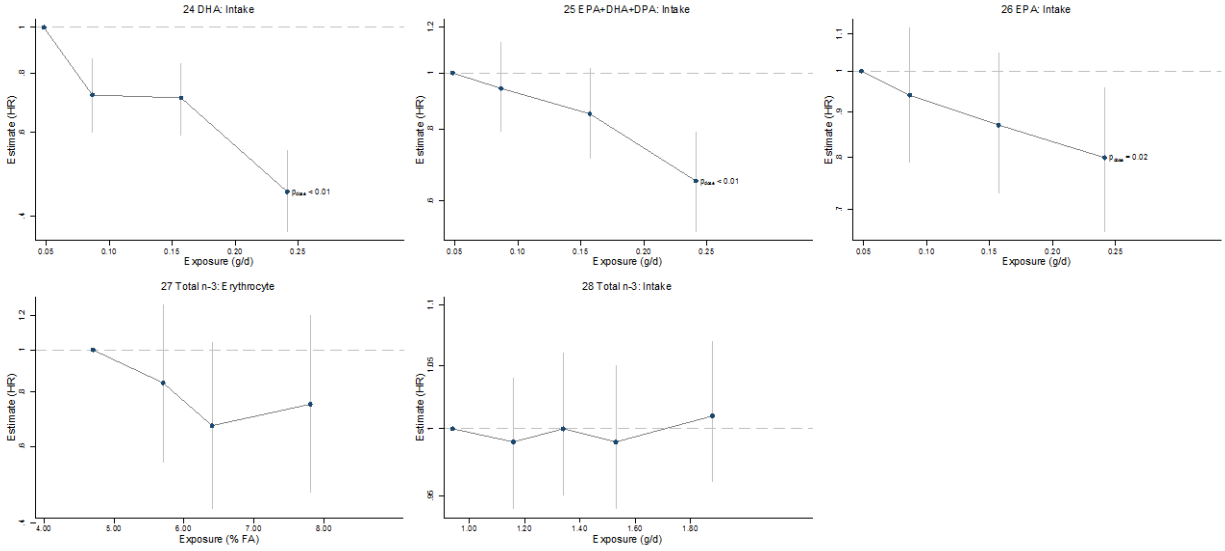
No trial evaluated incident HTN.

Observational Studies

Two studies evaluated the associations between intake of multiple n-3 FA or erythrocyte FA and new-onset hypertension after about 13 or 20 years of followup in health adults (**Appendix Table Y.3, Figure Y.4**).[177, 183] Statistically significant associations were found for younger, but not older, adults (with one exception). The Women's Health Study found that

overall total n-3 FA intake and erythrocyte levels were not significantly associated with risk of hypertension (plots #27 & 28). Among women 55 to 89 years old at baseline, there were also no significant associations with ALA, DHA, and EPA intake, and with erythrocyte total n-3 FA, ALA, DPA, and DHA levels, but higher erythrocyte EPA levels were associated with lower hypertension incidence. Among younger women, 39 to 54 years old at baseline, higher DHA intake and higher erythrocyte total n-3 FA, DPA, and DHA levels , but not ALA or EPA levels, were associated with lower HTN risk. Similarly, the CARDIA study, all in 18 to 30 year old adults, with 20 year followup, higher EPA (plot #26), DHA (plot #24), and EPA+DHA+DPA (plot #25) intake were all significantly associated with lower hypertension incidence.

Figure Y.4. n-3 FA associations with incident hypertension: Observational studies



Blood Pressure, Systolic and Diastolic

Randomized Controlled Trials

Twenty-eight RCTs provided data on effect of n-3 FA on systolic BP (**Table AA.1**). Twenty-seven RCTs provided data on effect of n-3 FA on diastolic BP (**Table AB.1**).

Total n-3 FA vs. placebo

Two RCTs evaluated supplementation with combined ALA and marine oil (1.2 or 2 g ALA, and 3.6 or 0.4 g EPA+DHA) versus placebo in people with at least one of several risk factors for CVD in one trial[110] or with CVD in the second trial.[114] In the at-risk population, at 1 month followup, no differences in systolic or diastolic BP were seen, with wide confidence intervals (systolic net change = −1.1 mmHg; 95% CI −44, 42; diastolic net change = −2.5 mmHg; 95% CI −31, 26). In the CVD population nonsignificant increases in systolic (2.3 mmHg; 95% CI −0.1, 4.6) and diastolic (0.5 mmHg; 95% CI −0.7, 1.7) BPs.

Marine oil vs. placebo

Twenty-five RCTs compared marine oil versus placebo (or control) and reported on changes in systolic BP in populations of healthy people, those at risk for CVD primarily related to a diagnosis of hypertension, and those with existing CVD (Table AA.1, **Figure AA.2**).[56, 62, 64, 73, 77, 78, 82, 88, 90, 91, 97, 101, 110, 114, 122, 142, 149, 153-155, 157, 161, 168, 169, 176] Across the 25 trials, no significant effect was found on systolic BP: summary net change = 0.27 mmHg (95% CI −0.30, 0.83). All but one of these trials also reported diastolic BP.[161] Across the 24 trials (Table AB.1, **Figure AB.2**), no significant effect was found on diastolic BP: summary net change = −0.19 mmHg (95% CI −0.52, 0.13).

Healthy population

Seven RCTs contributed to a pooled analysis of marine oils (EPA+DHA) against placebo for systolic BP, comprising data from 1170 healthy individuals with mean baseline systolic BP ranging between 107 to 126 mmHg.[64, 82, 90, 91, 149, 155, 157] One study compared both EPA (3.8 g/d) and DHA (3.6 g/d), separately, to placebo;[91] all other evaluated supplements with both EPA+DHA. Marine oil dosage ranged from 0.8 to 3.8 g/d, and follow-up duration from 2 months to 1 year. Five studies reported their compliance verification methods (including self-report, food records, pill count, and plasma measurement). All RCTs found no significant effect of EPA+DHA on systolic BP; net systolic BP varied between −3.0 and 1.2 mmHg. The pooled effect size was a nonsignificant −1.06 mmHg (95% CI −3.43, 1.31).

The same seven RCTs contributed to a pooled analysis of marine oils (EPA+DHA) against placebo for diastolic BP, comprising data from 1170 healthy individuals with mean baseline diastolic BP ranging between 65 to 81 mmHg.[64, 82, 90, 91, 149, 155, 157] All RCTs found no significant effect of EPA+DHA on diastolic BP; net diastolic BP varied between −1.0 and 0.6 mmHg. The pooled effect size was a nonsignificant −0.37 mmHg (95% CI −1.11, 0.38).

At risk for CVD population

Twelve RCTs contributed to a pooled analysis for systolic BP of marine oils (EPA+DHA) against placebo in those at risk for CVD, comprising data from 27,250 individuals, primarily due to hypertension, with mean baseline systolic BP ranging between 120 and 146 mmHg.[56, 73, 77, 78, 97, 101, 110, 122, 142, 153, 161, 169] One study compared DHA (2 g/d) to placebo;[97] the rest evaluated supplements with EPA+DHA. Dosage ranged from 0.30 to 6 g/d, and follow-up duration from 1 month to 6 years. Eight RCTs reported their compliance verification methods (including self-report, pill count, and plasma measurements). Across trials, the net change in systolic BP varied from −5.3 and 3.8, all of which were nonsignificant effects. The pooled effect size was a nonsignificant 0.43 (95% CI −0.39, 1.25).

Eleven RCTs contributed to a pooled analysis for diastolic BP of marine oils (EPA+DHA) against placebo in those at risk for CVD, comprising data from 27,212 individuals, primarily due to hypertension, with mean baseline diastolic BP ranging between 76 and 85 mmHg.[56, 73, 77, 78, 97, 101, 110, 122, 142, 153, 169] Across trials, the net change in diastolic BP varied from −4.5 and 0.7, all of which were nonsignificant effects. The pooled effect size was a nonsignificant −0.51 (95% CI −1.26, 0.24).

CVD population

Five RCTs contributed to a pooled analysis for systolic BP of marine oils (EPA+DHA) against placebo, comprising data from 9580 individuals with CVD (mean baseline systolic BP

130 to 142 mmHg).[62, 88, 114, 155, 176] A sixth trial reported only that no significant effect on BP was found.[168] Dosage ranged from 0.36 to 6 g/d, and follow-up durations from 1 and 4.7 years. They reported a variety of compliance verification methods (self-report, dietary questionnaire, pill count/audit, and plasma measurements). None of the RCTs found a significant effect of EPA+DHA on systolic BP, with net change ranging from −1 to 1.7 mmHg. The pooled effect size was a nonsignificant 0.34 (95% CI −0.29, 0.97).

The same five RCTs contributed to a pooled analysis for diastolic BP of marine oils (EPA+DHA) against placebo (mean baseline diastolic BP 77 to 83 mmHg).[62, 88, 155, 176] None of the RCTs found a significant effect of EPA+DHA on diastolic BP, with net change ranging from −0.5 to 1.0 mmHg. The pooled effect size was a nonsignificant −0.04 (95% CI −0.46, 0.37).

RCT subgroup analyses

Carter 2012 found no differences in effect on BP between two subpopulations of those with prehypertension or normal BP.[64]

By meta-regression, no differences in effect were found based on population (at risk P=0.74 systolic, P=0.31 diastolic; CVD P=0.88 systolic, P=0.40 diastolic), n-3 FA dose (P=0.64 systolic, P=0.26 diastolic), baseline systolic BP (P=0.60 systolic) or diastolic BP (P=0.78 diastolic), or followup duration (P=0.98 systolic, P=0.51 diastolic).

ALA vs. placebo

Five trials compared ALA supplementation to placebo, one in a healthy population,[82] three in at risk populations,[51, 110, 152] and one in a population with CVD (Tables AA.1 and AB.1).[114] The trials evaluated ALA doses ranging from 1.38 to 5.9 g/d; Jones 2014 evaluated these two doses of ALA versus placebo.[110] Followup ranged from 1 to 40 months. Compliance was confirmed in four trials and was >90 percent in one (Finnegan 2003). All four trials found no significant effect of ALA supplementation on systolic BP, ranging from −7.3 to 5.2 mmHg, or on diastolic BP, ranging from −7.3 to 1.0 mmHg, mostly with wide confidence intervals.

RCT subgroup analyses

Rodriguez-Leyva 2013 also found no differences in effect on systolic or diastolic BP in a subpopulation with systolic hypertension (>140 mmHg) compared with the study population as a whole.[152]

Marine oil, comparison of different doses

Three trials directly compared different doses of EPA+DHA, two in healthy populations,[82, 157] one in an at risk population (Tables AA.1 and AB.1).[166] All found no differences in effects on systolic or diastolic BP between higher and lower EPA+DHA doses (1.7 vs. 0.8 g/d; 1.8 vs. 0.9 or 0.45 g/d; 3.4 vs. 1.7 g/d).

ALA, comparison of different doses

One trial directly compared different doses of ALA (1.38 and 5.9 g/d) in an at risk population (Tables AA.1 and AB.1).[110] No differences in effects on systolic or diastolic BP were found, with wide confidence intervals, between higher and lower ALA doses (5.9 vs. 1.4 g/d).

Marine oils, comparison of different specific n-3 FA

Grimsgaard 1998 directly compared EPA 3.8 g/d and DHA 3.6 g/d supplementation, finding no differences in effect at 2 months (Tables AA.1 and AB.1).[91] Tatsuno 2013 compared two doses of EPA+DHA (3.4 and 1.7 g/d) and EPA 1.8 g/d; they did not report full data but stated there were no "clinically relevant changes" at 1 year.[166]

Marine oil vs. ALA

Finnegan 2003 compared two doses of EPA+DHA (1.7 and 0.8 g/d) and ALA 4.5 g/d in a healthy population.[82] (The study also tested ALA 9 g/d but that dose is excluded here because it does not meet eligibility criteria.) The comparisons between either dose of EPA+DHA and ALA found no differences in effect on systolic or diastolic BP at 4 months (Tables AA.1 and AB.1). Kromhout 2010 also compared EPA+DHA 0.4 g/d to ALA 2 g/d in a population with CVD. Neither systolic nor diastolic BP were significantly different between study arms.

Observational Studies

Observational studies did not evaluate systolic or diastolic BP.

Table AA.1. Systolic blood pressure: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Total n-3 FA vs. Placebo													
Jones 2014 24829493 Canada	At risk	ALA + EPA+DHA	3.48 DHA g/d+1.2 g/d ALA+0.12 EPA g/d+1.44 g/d DPA (suppl: CanolaDHA)	Placebo	0	1 mo	nd	130	120.62	130	120.62	−1.1 (−43.9, 41.8)	nd
Kromhout 2010 20929341 Netherlands	CVD	ALA + EPA+DHA	0.4 g/d EPA+DHA; 2 g/d ALA (Marine oil, plant oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1212	140.9	1236	141.9	2.3 (-0.1, 4.6)	NS
Marine oil vs. Placebo													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/day (suppl: marine oil)	Placebo	0	2 mo	nd	75	123.2	77	122.2	−1.2 (−2.9, 0.5)	nd
	Healthy	DHA	3.6 g/day (suppl: marine oil)	Placebo	0	2 mo	nd	72	121.3	77	122.2	−0.2 (−1.8, 1.4)	nd
Harrison 2004 15853118 UK	At risk	DHA	2 g/d (supp: marine oil)	Placebo	0	1.25 mo	Food diaries, biomarker check	101	130.9	112	134.7	−0.94% (−4.68%, 2.79%)	nd
Carter 2012 22707560 US	Healthy (normo-tensive)	EPA+DHA	1.6 EPA g/d+1.1 DHA g/d (suppl: marine oil)	Placebo	0	2 mo	Pill diary	19	110	19	107	−3 (−7, 1)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
	Healthy (pre-hypertensive)	EPA+DHA	1.6 EPA g/d+1.1 DHA g/d (suppl: marine oil)	Placebo	0			15	127	14	126	1 (−4.2, 6.2)	nd
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (suppl: marine oil, diet: marine oil margarine	Placebo	0	4 mo	Pill count, plasma measurement	31	118.4	30	123.2	0.2 (-5.6, 6.1)	nd
	Healthy	EPA+DHA	0.8 g/d (suppl: marine oil)	Placebo	0			30	119.6	30	123.2	2.8 (-4.1, 9.8)	nd
Grieger 2014 24454276 Australia	Healthy	EPA+DHA	0.8 g/d (diet: fish)	Low n−3 diet (usual diet)	0.017 g/d EPA and 0.004 g/d DHA (diet)	2 mo	Food Records	43	126	37	126	−2.0 (−9.3, 5.3)	nd
Rasmussen 2006 16469978 Europe and Australia	Healthy	EPA+DHA	2.4 g/d EPA+DHA	Placebo	0	3 mo	nd	80	122.6	82	122.3	−0.4 (−2.6, 1.8)	nd
Sacks 1994 8021472 US	Healthy	EPA+DHA	1.44 EPA g/d+0.96 DHA g/d+0.6 DPA g/d (suppl: marine oil)	Placebo	0	6 mo	FA measurement	175	122.9	175	122.6	1.2 (−0.3, 2.8)	NS
Sanders 2011 21865334 UK	Healthy	EPA+DHA	1.8 g/d (suppl: marine oil) EPA:DHA : 1.51	Placebo	0	1 y	Pill Count, Plasma Check	80	119.1	71	122.6	−0.3 (−4.3, 3.7)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
		EPA+DHA	0.9 g/d (suppl: marine oil) EPA:DHA : 1.51	Placebo	0			79	123.5	71	122.6	−0.8 (−4.8, 3.2)	nd
		EPA+DHA	0.45 g/d (suppl: marine oil) EPA:DHA : 1.51	Placebo	0			80	122.6	71	122.6	0 (−4, 4)	nd
Bosch 2012 22686415 Canada	At risk	EPA+DHA	EPA+DHA 0.84 g/d (suppl: marine oil)	Placebo	0	6 y	nd	6281	145.6	6255	146.0	0.1 (−0.6, 0.9)	nd
Tierney 2011 20938439 Northern Europe	At risk	EPA+DHA	EPA 0.26 g/d, DHA 0.19 g/d (suppl) [E:D 1.5]	Placebo	0	3 mo	Pill Count and plasma FA	100	137.73	106	139.53	0.1 (−4, 4.2)	NS
Derosa 2009 19397392 Italy	At risk	EPA+DHA	0.9 g/d EPA+1.5 g/d DHA (suppl: marine oil) E:D : 0.6	Placebo	0	6 mo	Pill Count	168	128.4	165	129.6	0 (−1.4, 1.4)	nd
Ebrahimi 2009 19593941 Iran	At risk	EPA+DHA	0.18 g/d EPA+0.12 g/d DHA (suppl: marine oil)	Placebo	0	6 mo	nd	47	130.7	42	123.6	−5.3 (−13.5, 2.9)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Einvik 2010 20389249 Norway	At risk	EPA+DHA (no diet intervention)	2.4 g n−3 fatty acids (1.17 g EPA and 0.84 g DHA) (Suppl: marine oil), E:D: 2:1	Placebo (no diet intervention)	0	3 y	Pharmacy records of remaining capsules, and measurements of serum n−3 PUFA	70	143	71	142	1 (−5.4, 7.4)	nd
		EPA+DHA (diet intervention)	2.4 g n−3 fatty acids (1.17 g EPA and 0.84 g DHA) (Suppl: marine oil), E:D: 2:1	Placebo (diet intervention)	0			69	141	68	143	3 (−3.5, 9.5)	nd
Holman 2009 19002433 UK	At risk	EPA+DHA (+/− atorvastatin)	EPA+DHA 1.68 g/d (suppl: marine oil) E:D : 1.2	Placebo (+/- atorvastatin)	0	4 mo	Lab results	371	145	361	148	0.4 (−1.9, 2.7)	nd
Lungershausen 1994 7852747 Australia	At risk	EPA+DHA	1.9 g/d EPA, 1.5 g/d DHA (suppl) E:D : 1.27	Placebo	0	1.5 mo	Interview and Pill Count	42	132	42	132	−3.1 (−8.3, 2.1)	nd
Nodari 2011 21215550 Italy	At risk	EPA+DHA	4.25 − 4.41 g/d EPA+DHA daily for the first month followed by 1.7 – 1.764 g/d (suppl: marine oil) EPA:DHA : 0.6	Placebo	0	1 y	nd	67	119	66	120	3 (−0.4, 6.4)	0.015

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	EPA+DHA <0.85 g/d (suppl: marine oil) (E:D 1]	Placebo	0	5 y	Patient Self−Report	6244	140.3	6269	140.1	0.2 (−0.4, 0.7)	0.57
Soares 2014 24652053 Brazil	At risk	EPA+DHA (and dietary intervention)	3 g/d (suppl: marine oil)	Placebo and dietary intervention	0	3 mo	nd	20	130.2	18	134.4	0.6 (−1.5, 2.7)	0.702 (overall)
		EPA+DHA (and dietary intervention+exercise)	3 g/d (suppl: marine oil)	Placebo and dietary intervention and exercise	0			17	131.6	15	131.1	3.8 (1.2, 6.4)	0.702 (overall)
Jones 2014 24829493 Canada	At risk	EPA+DHA+ALA (Canola DHA)	3.48 DHA g/d+1.2 g/d ALA+0.12 EPA g/d+1.44 g/d DPA (suppl: CanolaDHA)	ALA (Canola Oleic)	1.38 g/d	1 mo	nd	130	120.62	130	120.62	−1.2 (−44, 41.7)	nd
Burr 1989 2571009 UK	CVD	EPA+DHA	0.357 EPA g/d+nd DPA (suppl: marine oil, diet: fish)	No intervention	0	2 y	Dietary Questionnaire	1015	129.7	1018	130.1	0.40 (−1.33, 2.13)	nd
Galan 2010 21115589 France	CVD	EPA+DHA (+/− B vitamin)	0.6 g/d (suppl: marine oil) [E:D 2:1]	Placebo (+/− B vitamin	0	4.7 y	Self−Report	1253	134	1248	133	−0.06 (−0.9, 0.8)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	EPA+DHA 0.75 – 0.882 g/d (suppl: marine oil) (E:D : 0.833]	Placebo	0	3.9 y	Pill count	3494	126	3481	126	nd	0.47
Sacks 1995 7759696 US	CVD	EPA+DHA	2.88 g/d EPA and 3.12 g/d DHA (suppl: marine oil) (E:D 0.923)	Placebo	0	2.4 y	Pill Count	31	126	28	133	−1.0 (−14, 12.0)	nd
von Schacky 1999 10189324 Canada	CVD	EPA+DHA	EPA+DHA 3.3 g/d for 3 months then 1.65 g/d for 21 months (suppl: marine oil)	Placebo	0	1 y	Interogation, Pill Count, and analysis of FA	112	132.0	111	129.6	−0.1 (−5.0, 4.8)	NS
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1192	142.3	1236	141.9	1.7 (-0.6, 3.9)	NS
		EPA+DHA (+ALA)	0.4 g/d (Marine oil) [E:D 3:2]	(ALA)	0			1212	140.9	1197	141.4	0.2 (-2.0, 2.5)	nd
EPA+DHA vs. EPA+DHA (doses)													
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d	EPA+DHA	0.8 g/d	4 mo	Pill count, plasma measurement	31	118.4	30	119.6	-2.6 (-7.9, 2.7)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Sanders 2011 21865334 UK	Healthy	EPA+DHA	1.8 g/d (suppl: marine oil) EPA:DHA : 1.51	EPA+DHA	0.9 g/d (suppl: marine oil)	1 y	Pill Count, Plasma Check	80	119.1	79	123.5	0.5 (−3.5, 4.5)	nd
		EPA+DHA	1.8 g/d (suppl: marine oil) EPA:DHA : 1.51	EPA+DHA	0.45 g/d (suppl: marine oil)			80	119.1	80	122.6	−0.3 (−4.3, 3.7)	nd
Tatsuno 2013 24314359 Japan	At risk	EPA+DHA	EPA+DHA 3.36 g/d (suppl: marine oil) E:D 1.24	EPA+DHA	EPA+DHA 1.68 g/d (suppl: marine oil) E:D 1.24	1 y	nd	171	nd	165	nd	1.6 (nd)	nd
Marine oil vs. marine oil (miscellaneous)													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl: marine oil)	DHA	3.6 g/day (suppl: marine oil)	2 mo	nd	77	122.2	72	121.3	−1.0 (−2.8, 0.8)	nd
Tatsuno 2013 24314359 Japan	At risk	EPA+DHA	EPA+DHA 3.36 g/d (suppl: marine oil)E:D 1.24	EPA	1.8 g/d (suppl: marine oil)	1 y	nd	171	nd	167	nd	2.6 (nd)	nd
		EPA+DHA	EPA+DHA 1.68 g/d (suppl: marine oil) E:D 1.24	EPA	1.8 g/d (supp: marine oil)			165	nd	167	nd	1.0 (nd)	nd
ALA vs. Placebo													

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Finnegan 2003 12663273 UK	Healthy	ALA	4.5 g/d (rapeseed oil margarine)	Placebo	0	4 mo	Return of margarine pots (>90%)	30	118.2	30	123.2	4.5 (-0.6, 9.6)	nd
Rodriguez-Leyva 2013 24126178 Canada	At risk	ALA	5.9 g/day (flaxseed)	Placebo	0	6 mo	Plasma ALA and enterolignan levels	45	143.3	42	142.4	−7.3 (−15.4, 0.8)	nd
Baxheinrich 2012 22894911 Germany	At risk	ALA	3.46 g/day (suppl: plant oil)	Placebo	0	6 mo	Dietary records	40	142.4	41	140.1	−1.8 (−8.3, 4.7)	0.026
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/day (canola)	Placebo	0	1 mo	nd	130	120.62	130	120.62	−1.1 (−43.9, 41.8)	nd
		ALA	1.38 g/d (canola)	Placebo	0			130	120.62	130	120.62	0.1 (−42.8, 42.9)	nd
Kromhout 2010 20929341 Netherlands	CVD	ALA	2 g/d (plant oil)	Placebo	0	40 mo	Audit of unused margarine tubs returned	1197	141.4	1236	141.9	2.1 (-0.2, 4.3)	NS
		ALA (+EPA+DHA)	2 g/d (plant oil)	(EPA+DHA)	0			1212	140.9	1192	142.3	0.6 (-1.6, 2.9)	nd
ALA vs. ALA (doses)													
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/day (canola)	ALA	1.38 g/d (canola)	1 mo	nd	130	120.62	130	120.62	−1.2 (−44, 41.7)	nd
Marine oil vs. ALA													
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d	ALA	4.5 g/d (rapeseed oil margarine)			31	118.4	30	118.2	-4.3 (-9.4, 0.9)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
		EPA+DHA	0.8 g/d	ALA	4.5 g/d (rapeseed oil margarine)			30	119.6	30	118.2	-1.7 (-6.1, 2.8)	nd
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	ALA	2 g/d (plant oil)	40 mo	Audit of unused margarine tubs returned	1192	142.3	1197	141.4	-0.4 (-2.6, 1.8)	nd

Figure AA.2. Systolic blood pressure: Randomized trials of marine oils

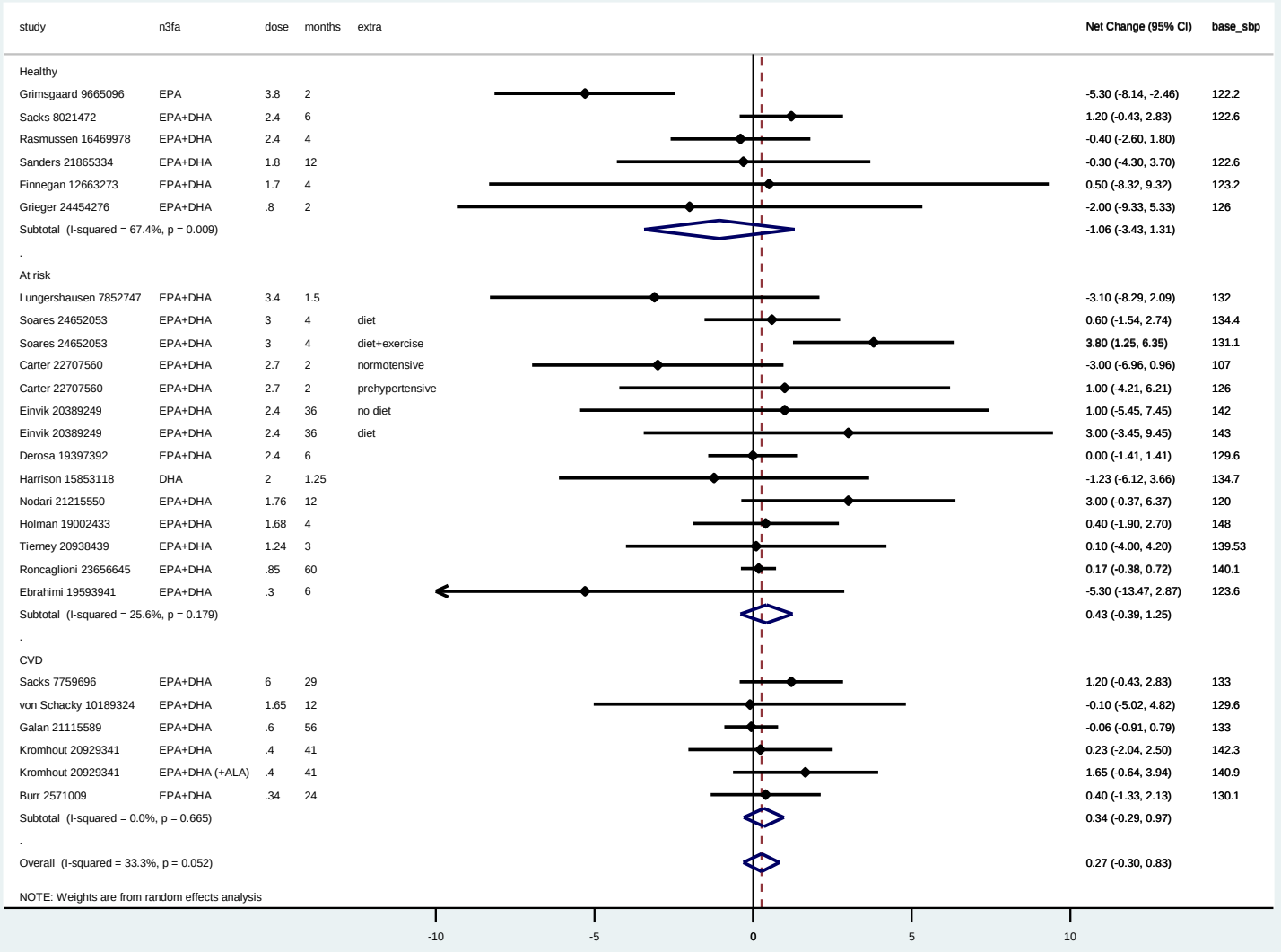


Table AB.1. Diastolic blood pressure: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Total n-3 FA vs. Placebo													
Jones 2014 24829493 Canada	At risk	ALA + EPA+DHA	3.48 DHA g/d+1.2 g/d ALA+0.12 EPA g/d+1.44 g/d DPA (suppl: CanolaDHA)	Placebo	0	1 mo	nd	130	77.04	130	77.04	−2.5 (−31.3, 26.3)	nd
Kromhout 2010 20929341 Netherlands	CVD	ALA + EPA+DHA	0.4 g/d EPA+DHA; 2 g/d ALA (Marine oil, plant oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1212	nd	1236	nd	0.5 (-0.7, 1.7)	NS
Marine oil vs. Placebo													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/day (suppl: marine oil)	Placebo	0	2 mo	nd	75	78.1	77	76.9	-0.6 (-1.9, 0.7)	nd
	Healthy	DHA	3.6 g/day (suppl: marine oil)	Placebo	0	2 mo	nd	72	76.1	77	76.9	-0.4 (-1.8, 1.0)	nd
Harrison 2004 15853118 UK	At risk	DHA	2 g/d (supp: marine oil)	Placebo	0	1.25 mo	Food diaries, biomarker check	101	81.1	112	81.8	−2.19% (−5.57%, 1.18%)	nd
Carter 2012 22707560 US	Healthy (normo-tensive)	EPA+DHA	1.6 EPA g/d+1.1 DHA g/d (suppl: marine oil)	Placebo	0	2 mo	Pill diary	19	66	19	65	−1.0 (−3.6, 1.6)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
	Healthy (pre-hypertensive)	EPA+DHA	1.6 EPA g/d+1.1 DHA g/d (suppl: marine oil)	Placebo	0			15	68	14	74	0 (−5.2, 5.2)	nd
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (suppl: marine oil, diet: marine oil margarine	Placebo	0	4 mo	Pill count, plasma measurement	31	74.8	30	76.0	-0.1 (-5, 4.7)	nd
	Healthy	EPA+DHA	0.8 g/d (suppl: marine oil)	Placebo	0			30	74.6	30	76.0	1.9 (-3.7, 7.6)	nd
Grieger 2014 24454276 Australia	Healthy	EPA+DHA	0.8 g/d (diet: fish)	Low n−3 diet (usual diet)	0.017 g/d EPA and 0.004 g/d DHA (diet)	2 mo	Food Records	43	69	37	67	0 (−4.8, 4.8)	nd
Rasmussen 2006 16469978 Europe and Australia	Healthy	EPA+DHA	2.4 g/d EPA+DHA	Placebo	0	3 mo	nd	80	76	82	77	−0.6 (−2.8, 0.8)	nd
Sacks 1994 8021472 US	Healthy	EPA+DHA	1.44 EPA g/d+0.96 DHA g/d+0.6 DPA g/d (suppl: marine oil)	Placebo	0	6 mo	FA measurement	175	81.0	175	81.0	−0.5 (−1.5, 0.5)	NS
Sanders 2011 21865334 UK	Healthy	EPA+DHA	1.8 g/d (suppl: marine oil) EPA:DHA : 1.51	Placebo	0	1 y	Pill Count, Plasma Check	80	71.8	71	74.1	0.6 (−1.4, 2.6)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
		EPA+DHA	0.9 g/d (suppl: marine oil) EPA:DHA : 1.51	Placebo	0			79	73.9	71	74.1	0.6 (−1.5, 2.7)	nd
		EPA+DHA	0.45 g/d (suppl: marine oil) EPA:DHA : 1.51	Placebo	0			80	71.2	71	74.1	1.2 (−0.9, 3.3)	nd
Bosch 2012 22686415 Canada	At risk	EPA+DHA	EPA+DHA 0.84 g/d (suppl: marine oil)	Placebo	0	6 y	nd	6281	84.1	6255	84.2	0.1 (−0.3, 0.5)	nd
Tierney 2011 20938439 Northern Europe	At risk	EPA+DHA	EPA 0.26 g/d, DHA 0.19 g/d (suppl) [E:D 1.5]	Placebo	0	3 mo	Pill Count and plasma FA	100	85.5	106	85.52	0.7 (−1.7, 3.1)	NS
Derosa 2009 19397392 Italy	At risk	EPA+DHA	0.9 g/d EPA+1.5 g/d DHA (suppl: marine oil) E:D : 0.6	Placebo	0	6 mo	Pill Count	168	80.6	165	81.4	0.2 (−1.3, 1.7)	nd
Ebrahimi 2009 19593941 Iran	At risk	EPA+DHA	0.18 g/d EPA+0.12 g/d DHA (suppl: marine oil)	Placebo	0	6 mo	nd	47	81.7	42	78.3	−4.5 (−9, 0)	nd
Einvik 2010 20389249 Norway	At risk	EPA+DHA (no diet intervention)	2.4 g n−3 fatty acids (1.17 g EPA and 0.84 g DHA) (Suppl: marine oil), E:D: 2:1	Placebo (no diet intervention)	0	3 y	Pharmacy records of remaining capsules, and measurements of serum n−3 PUFA	63	83	56	83	0 (−3.9, 3.9)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
		EPA+DHA (diet intervention)	2.4 g n−3 fatty acids (1.17 g EPA and 0.84 g DHA) (Suppl: marine oil), E:D: 2:1	Placebo (diet intervention)	0			59	85	58	83	−1.1 (−5, 3)	nd
Holman 2009 19002433 UK	At risk	EPA+DHA (+/− atorvastatin)	EPA+DHA 1.68 g/d (suppl: marine oil) E:D : 1.2	Placebo (+/- atorvastatin)	0	4 mo	Lab results	371	81	361	82	0.6 (−1.9, 2.7)	nd
Lungershausen 1994 7852747 Australia	At risk	EPA+DHA	1.9 g/d EPA, 1.5 g/d DHA (suppl) E:D : 1.27	Placebo	0	1.5 mo	Interview and Pill Count	42	76.2	42	76.2	−1.8 (−4.8, 1.2)	nd
Nodari 2011 21215550 Italy	At risk	EPA+DHA	4.25 − 4.41 g/d EPA+DHA daily for the first month followed by 1.7 – 1.764 g/d (suppl: marine oil) EPA:DHA : 0.6	Placebo	0	1 y	nd	67	76	66	76	−1.0 (−2.6, 0.6)	0.015
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	EPA+DHA <0.85 g/d (suppl: marine oil) (E:D 1]	Placebo	0	5 y	Patient Self−Report	6239	82.9	6266	82.5	−0.2 (−25, 24.6)	0.57
Burr 1989 2571009 UK	CVD	EPA+DHA	0.357 EPA g/d+nd DPA (suppl: marine oil, diet: fish)	No intervention	0	2 y	Dietary Questionnaire	1015	79.3	1018	80.2	0.19 (−0.88, 1.26)	nd

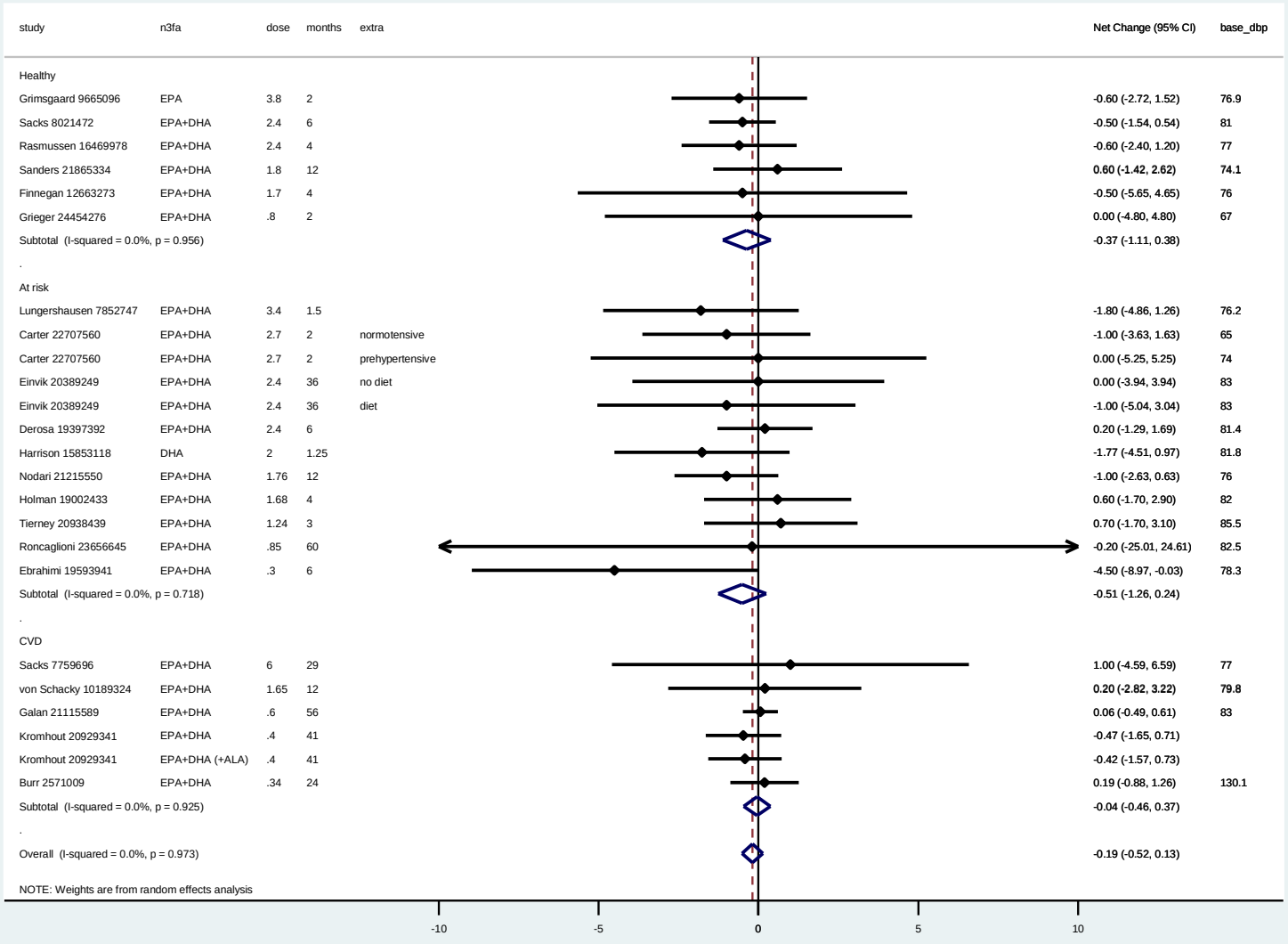
Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Galan 2010 21115589 France	CVD	EPA+DHA (+/− B vitamin)	0.6 g/d (suppl: marine oil) [E:D 2:1]	Placebo (+/− B vitamin	0	4.7 y	Self−Report	1253	84	1248	83	0.06 (−0.5, 0.6)	nd
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	EPA+DHA 0.75 – 0.882 g/d (suppl: marine oil) (E:D : 0.833]	Placebo	0	3.9 y	Pill count	3494	77	3481	77	nd	0.43
Sacks 1995 7759696 US	CVD	EPA+DHA	2.88 g/d EPA and 3.12 g/d DHA (suppl: marine oil) (E:D 0.923)	Placebo	0	2.4 y	Pill Count	31	76	28	77	1 .0(−4.6, 6.6)	nd
von Schacky 1999 10189324 Canada	CVD	EPA+DHA	EPA+DHA 3.3 g/d for 3 months then 1.65 g/d for 21 months (suppl: marine oil)	Placebo	0	1 y	Interogation, Pill Count, and analysis of FA	112	80.7	111	79.8	0.2 (−2.8, 3.2)	NS
Jones 2014 24829493 Canada	At risk	ALA + EPA+DHA (Canoal DHA)	3.48 DHA g/d+1.2 g/d ALA+0.12 EPA g/d+1.44 g/d DPA (suppl: CanolaDHA)	ALA (Canola Oleic)	1.38 g/d	1 mo	nd	130	77.04	130	77.04	−2.2 (−38.1, 33.8)	nd
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1192	nd	1236	nd	-0.4 (-1.6, 0.7)	NS

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
		EPA+DHA (+ALA)	0.4 g/d (Marine oil) [E:D 3:2]	ALA	0			1212	nd	1197	nd	-0.5 (-1.6, 0.7)	nd
EPA+DHA vs. EPA+DHA (doses)													
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d	EPA+DHA	0.8 g/d	4 mo	Pill count, plasma measurement	31	74.8	30	74.6	-2.1 (-6.6, 2.4)	nd
Sanders 2011 21865334 UK	Healthy	EPA+DHA	1.8 g/d (suppl: marine oil) EPA:DHA : 1.51	EPA+DHA	0.9 g/d (suppl: marine oil)	1 y	Pill Count, Plasma Check	80	71.8	79	73.9	0 (−2.0, 2.0)	nd
		EPA+DHA	1.8 g/d (suppl: marine oil) EPA:DHA : 1.51	EPA+DHA	0.45 g/d (suppl: marine oil)			80	71.8	80	71.2	−0.6 (−2.5, 1.3)	nd
Tatsuno 2013 24314359 Japan	At risk	EPA+DHA	EPA+DHA 3.36 g/d (suppl: marine oil) E:D 1.24	EPA+DHA	EPA+DHA 1.68 g/d (suppl: marine oil) E:D 1.24	1 y	nd	171	nd	165	nd	0.4 (nd)	nd
Marine oil vs. marine oil (miscellaneous)													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl: marine oil)	DHA	3.6 g/day (suppl: marine oil)	2 mo	nd	77	78.1	72	76.1	-0.2 (-1.6, 1.2)	nd
Tatsuno 2013 24314359 Japan	At risk	EPA+DHA	EPA+DHA 3.36 g/d (suppl: marine oil)E:D 1.24	EPA	1.8 g/d (suppl: marine oil)	1 y	nd	171	nd	167	nd	−0.8 (nd)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
		EPA+DHA	EPA+DHA 1.68 g/d (suppl: marine oil) E:D 1.24	EPA	1.8 g/d (supp: marine oil)			165	nd	167	nd	−1.2 (nd)	nd
ALA vs. Placebo													
Finnegan 2003 12663273 UK	Healthy	ALA	4.5 g/d (rapeseed oil margarine)	Placebo	0	4 mo	Return of margarine pots (>90%)	30	76.0	30	76.0	0.6 (-3.5, 4.7)	nd
Rodriguez-Leyva 2013 24126178 Canada	At risk	ALA	5.9 g/day (flaxseed)	Placebo	0	6 mo	Plasma ALA and enterolignan levels	45	77	42	79	−7.3 (−15.4, 0.8)	nd
Baxheinrich 2012 22894911 Germany	At risk	ALA	3.46 g/day (suppl: plant oil)	Placebo	0	6 mo	Dietary records	40	91.8	41	90.2	−3.9 (−8.1, 0.3)	0.026
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/day (canola)	Placebo	0	1 mo	nd	130	77.04	130	77.04	0 (−28.8, 28.8)	nd
		ALA	1.38 g/d (canola)	Placebo	0			130	77.04	130	77.04	−0.3 (−36.2, 35.6)	nd
Kromhout 2010 20929341 Netherlands	CVD	ALA	2 g/d (plant oil)	Placebo	0	40 mo	Audit of unused margarine tubs returned	1197	nd	1236	nd	1.0 (-0.2, 2.1)	NS
		ALA (+EPA+DHA)	2 g/d (plant oil)	(EPA+DHA)	0			1212	nd	1192	nd	0.9 (-0.3, 2.1)	nd
ALA vs. ALA (doses)													
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/day (canola)	ALA	1.38 g/d (canola)	1 mo	nd	130	77.04	130	77.04	0.3 (−35.6, 36.2)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mmHg	Ctrl N	Ctrl Baseline, mmHg	Net Chg, mmHg	Reported P value
Marine oil vs. ALA													
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d	ALA	4.5 g/d (rapeseed oil margarine)			31	74.8	30	76.0	-0.7 (-5.3, 3.8)	nd
		EPA+DHA	0.8 g/d	ALA	4.5 g/d (rapeseed oil margarine)			30	74.6	30	76.0	1.3 (-2.4, 5.1)	nd
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	ALA	2 g/d (plant oil)	40 mo	Audit of unused margarine tubs returned	1192	nd	1197	nd	-1.4 (-2.5, -0.2)	nd

Figure AB.2. Diastolic blood pressure: Randomized trials of marine oils



Mean Arterial Blood Pressure

Randomized Controlled Trials

Four RCTs reported on mean arterial blood pressure (MAP), all of which evaluated only marine oils (Table AC.1).[64, 88, 91, 157]

Marine oil vs. placebo

Healthy population

Three trials evaluated healthy populations, including the previously described trial that compared EPA 3.8 g/d and DHA 3.6 g/d to placebo,[91] the trial of 2.7 g/d EPA+DHA in two healthy subgroups (with normotension or prehypertension),[64] and the comparison of 1.8 g/d, 0.9 g/d, and 0.45 g/d versus placebo.[157] Followup was either 2 months or 1 year. Baseline MAP ranged from 79 to 92 mmHg. All trials found no significant effect on MAP, with estimates of net change ranging from −1 to 2 mmHg.

CVD population

One trial of 0.6 g/d EPA+DHA versus placebo (with or without B vitamin) was conducted in 2501 people with a history of CVD.[88] At 4.7 years, there was no difference in MAP between the two groups.

RCT subgroup analyses

Carter 2012 found no differences in effect between two subpopulations of those with prehypertension or normal BP.[64]

Marine oil, comparison of different doses

One trial directly compared different doses of EPA+DHA in healthy populations.[157] Sanders 2011 found no differences in effects on MAP between higher and lower EPA+DHA doses (1.8, 0.9 or 0.45 g/d).

Marine oils, comparison of different specific n-3 FA

Grimsgaard 1998 directly compared EPA 3.8 g/d and DHA 3.6 g/d supplementation, finding no differences in effect at 2 months.[91]

Observational Studies

Observational studies did not evaluate systolic or diastolic BP.

Table AC.1. Mean arterial blood pressure: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL	Reported P value
Marine oil vs Placebo													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl=marine oil)	Placebo	0	2 mo	nd	75	92.9	77	91.8	-0.4 (-1.9, 1.1)	nd
		DHA	3.6 g/d (suppl=marine oil)	Placebo	0	2 mo	nd	72	90.6	77	91.8	0.4 (-1.3, 2.1)	nd
Carter 2012 22707560 US	Healthy (normotensive)	EPA+DHA	1.6 EPA g/d + 1.1 DHA g/d (suppl=marine oil)	Placebo	0	2 mo	Pill diary	19	80	19	79	−1 (−3.8, 1.8)	nd
	Healthy (prehypertensive)	EPA+DHA	1.6 EPA g/d + 1.1 DHA g/d (suppl=marine oil)	Placebo	0	2 mo	Pill diary	15	88	14	92	1 (−3.8, 5.8)	nd
Sanders 2011 21865334 UK	Healthy	EPA+DHA	1.8 g/d (suppl=marine oil)	Placebo	0	1 y	Pill Count, Plasma Check	80	91	71	93	2 (−1.4, 5.4)	nd
		EPA+DHA	0.9 g/d (suppl=marine oil)	Placebo	0	1 y	Pill Count, Plasma Check	79	94	71	93	1 (−2.4, 4.4)	nd
		EPA+DHA	0.45 g/d (suppl=marine oil)	Placebo	0	1 y	Pill Count, Plasma Check	80	93	71	93	−1 (−4.5, 2.5)	nd
Galan 2010 21115589 France	CVD	EPA+DHA (+/- B vitamin)	0.6 g/d (suppl=marine oil) [E:D 2:1]	Placebo (+/- B vitamin	0	4.7 y	Self-Report	1253	nd	1248	nd	0.007(nd)	NS
Marine oil vs Marine oil (doses)													

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL	Reported P value
Sanders 2011 21865334 UK	Healthy	EPA+DHA	1.8 g/d(suppl= marine oil)	EPA+DHA	0.9 g/d(suppl= marine oil)	1 y	Pill Count, Plasma Check	80	91	79	94	1 (−2.2, 4.2)	nd
		EPA+DHA	1.8 g/d(suppl= marine oil)	EPA+DHA	0.45 g/d(suppl= marine oil)	1 y	Pill Count, Plasma Check	80	91	80	93	3 (−0.4, 6.4)	nd
EPA vs DHA													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/day (suppl=marine oil)	DHA	3.6 g/day (suppl=marine oil)	2 mo	nd	75	92.9	72	90.6	-0.8 (-2.5, 0.9)	nd

Low Density Lipoprotein Cholesterol

Randomized Controlled Trials

Thirty-four RCTs provided data on effect of n-3 FA on low density lipoprotein cholesterol (LDL-c) (**Table AD.1**).[51, 56, 57, 63, 65, 66, 73, 77, 79, 82, 90, 91, 97, 101, 110, 111, 114, 121, 122, 124-126, 145, 149, 150, 153, 155, 159, 160, 168, 169, 171, 176, 189]

Total n-3 FA vs. placebo

Two trials compared total n-3 FA (ALA+EPA+DHA) versus placebo, following 2708 patients for 1 and 40 months; one in people at increased risk for CVD,[110] one in people with CVD.[114] Baseline LDL-c measurements were 100 and 129 mg/dL. Compliance was measured in both studies, but not reported. The trial in an at risk population found a statistically significant increase in LDL-c with combined ALA 1.2 g/d (canola oil) and EPA+DHA+DPA 5 g/d (6.6 mg/dL; 95% CI 0.5, 12.6).[110] The trial in a CVD population found no significant effect on LDL-c with ALA 2 g/d and EPA+DHA 0.4 g/d.[114]

Marine oil vs. placebo

Thirty-three trials evaluated the effect of marine oils versus placebo on LDL-c.[56, 57, 63, 65, 66, 73, 77, 79, 82, 90, 91, 97, 101, 110, 111, 114, 121, 122, 124-126, 145, 149, 150, 153, 155, 159, 160, 168, 169, 171, 176, 189] Doses of EPA+DHA±DPA ranged from 0.3 to 6 g/d (median 2.4 g/d) and followup time ranged from 1 month to 6 years (median 3 months). Across populations, the meta-analyzed summary net difference in LDL-c with EPA+DHA versus placebo (or equivalent) was a nonsignificant 0.3 mg/dL (95% CI −0.7, 1.2) (**Figure AD.2**).

Healthy population

Nine of the trials of marine oils versus placebo were conducted in healthy populations, comprising data from 1282 individuals with mean baseline LDL-c ranging from 100 to 218 and followup duration from 1 to 6 months.[63, 65, 66, 82, 90, 91, 97, 145, 149] Two studies compared both purified EPA (3.3 and 3.8 g/d) and DHA (3.6 and 3.7 g/d), separately, to placebo;[91, 145] all other evaluated supplements with both EPA+DHA, with doses ranging from 0.7 to 6 g/d. Compliance was verified with pill counts, dietary records, or biomarker confirmation in six of the studies. All but one RCT found no significant effect of EPA+DHA on LDL-c; net LDL-c varied between −5.4 and 12.7 mg/dL. The pooled effect size was a nonsignificant 0.8 mg/dL (95% CI −1.6, 3.2).

At risk for CVD population

Eighteen of the trials were conducted in populations at increased risk of CVD, comprising data from 30,026 individuals with mean baseline LDL-c ranging from 82 to 218 and followup duration from 1 month to 6 years.[56, 57, 73, 77, 97, 101, 110, 111, 121, 122, 124, 125, 153, 159, 160, 169, 171, 189] One study compared purified DHA (2 g/d) to placebo;[97] all other evaluated supplements with both EPA+DHA, with doses ranging from 0.3 to 6 g/d. Compliance was verified with pill counts, dietary records, self-report or biomarker confirmation in 11 of the studies. All but two RCTs found no significant effect of EPA+DHA on LDL-c; net change LDL-c varied between −7.5 and 6.6 mg/dL. The pooled effect size was a nonsignificant 0.3 mg/dL (95% CI −0.7, 1.3).

CVD population

Seven of the trials were conducted in people with CVD, comprising data from 20,743 individuals with mean baseline LDL-c ranging from 98 to 177 mg/dL and followup duration from 9 months to 3.9 years.[79, 114, 126, 150, 155, 168, 176] Compliance was verified in four of the studies, by pill count or equivalent. All trials found no significant effect on LDL-c; net change LDL-c varied from −0.8 to 5.8 mg/dL. The pooled effect size was a nonsignificant 0.4 mg/dL (95% CI −1.7, 2.6).

RCT subgroup analyses

Eight of the trials compared effects of marine oils in different subgroups of participants; five reported statin vs no statin,[63, 101, 114, 121, 171] one with or without vitamin C,[159] one men vs women,[65] one older vs younger age,[65] and one saturated FA diet vs monosaturated FA diet.[149] All found (or reported) no significant interactions (differences in effect) by subgroup or cointervention.

By meta-regression, across studies there were no significant differences in effect (interactions) by LDL-c baseline (P=0.09), n-3 FA dose (P=0.99), followup duration (P=0.72), or population (at risk P=0.65; CVD P=0.97).

Marine oil, comparison of different doses

Seven RCTs directly compared different doses of marine oils (EPA+DHA),[57, 65, 82, 111, 125, 166, 189] between 0.7 and 4 g/d. All comparisons were nonsignificant for effect on LDL-c, with estimates of differences ranging from −5 mg/dL (95% CI −18.8, 8.8; 4 vs. 2 g/d) to 12.7 mg/dL (95% CI −4.8, 30.2; 1.7 vs. 0.8 g/d).

ALA versus placebo

Four trials compared ALA to placebo (or equivalent) in a healthy population,[82] at-risk populations,[51, 110] and a population with CVD.[114] In total, there were 5368 participants followed for 1 to 40 months, with ALA doses of 1.4 to 5.9 g/d. None of the trials found a significant effect of ALA on LDL-c, with net changes ranging from −1.9 to 2.3 mg/dL, mostly with wide confidence intervals.

ALA, comparison of different doses

One trial compared ALA 5.9 and 1.4 g/d and found no difference in effect on LDL-c with wide confidence intervals (1.9 mg/dL; 95% CI −94, 97).[110]

Comparison of different specific n-3 FA

Two trials directly compared EPA (3.8 or 3.3 g/d) to DHA (3.6 or 3.7 g/d).[91, 145] Both found larger, but nonsignificant, relative reductions in LDL-C with EPA (−5.8 [95% CI −11.7, 0.1]; −6.2 [−21.8, 9.4]). One trial compared two doses of EPA+DHA (3.4 and 1.7 g/d) to EPA 1.8 g/d,[166] with no significant differences between marine oil formulations. Two trials compared EPA+DHA to ALA, one comparing two doses of EPA+DHA (1.7 and 0.8 g/d) to ALA 4.5 g/d,[82] one comparing 0.4 g/d EPA+DHA to 2 g/d ALA.[114] All comparisons were reported as nonsignificant, but the comparison of the higher dose marine oil in Finnegan 2003 found a large relative increase in LDL-c with a significant estimated CI (14.0 mg/dL; 95% CI 0.4, 27.7).

Observational Studies

Observational studies did not evaluate LDL-c.

Table AD.1. Low Density Lipoprotein Cholesterol: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Total n-3 FA vs Placebo													
Jones 2014 24829493 Canada	At risk	ALA + EPA+DHA	ALA: 1.2 g/d, EPA: 0.1 g/d, DHA: 3.5 g/d, DPA: 1.4 g/d (canola+DHA)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	129.34	130	129.34	6.6 (0.5, 12.6)	<0.05
Kromhout 2010 20929341 Netherlands	CVD	ALA + EPA+DHA	0.4 g/d EPA+DHA; 2 g/d ALA (Marine oil, plant oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1212	98	1236	100	0.8 (-2.4, 4.0)	NS
Marine oil vs Placebo													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl)	Placebo	0	2 mo	pill count	75	156.8	77	156.0	-5.4 (-11.3, 0.5)	nd
		DHA	3.6 g/d (suppl)	Placebo	0	2 mo	pill count	72	156.8	77	156.0	0.4 (-5.4, 6.2)	nd
Olano-Martin 2010 19748619 UK	Healthy	EPA	3.3 g/d (Marine oil)	Placebo	0	1 mo	nd	38	136.3	38	136.7	3.1 (-12, 18.2)	NS
		DHA	3.7 g/d (Marine oil)	Placebo	0	1 mo	nd	38	139.4	38	136.7	6.2 (-5.1, 17.5)	NS
Harrison 2004 15853118 Scotland, UK	At risk	DHA	2 g/d (food fortification)	Placebo	0	1.25 mo	Food diary (biomarker confirmation)	101	218	112	193	-7.5 (-15.9, 30.8)	
Carrepeiro 2011 21561620 Brazil	Healthy	EPA+DHA + Statin	2.4 g/d (Marine oil)	Placebo + Statin	0	6 mo	nd	20	133.4	20	116.9	-1.5 (-3.5, 0.4)	0.128
		EPA+DHA	2.4 g/d (Marine oil)	Placebo	0	6 mo	nd	23	136	23	144.5	-0.8 (-2.8, 1.2)	0.431
Caslake 2008 18779276 UK	Healthy	EPA+DHA	1.8 g/d (Marine oil)	Placebo	0	2 mo	Pill count	312	148.5	312	147.2	2.7 (-3.0, 8.4)	<0.017
		EPA+DHA	0.7 g/d (Marine oil)	Placebo	0	2 mo	Pill count	312	148.5	312	147.2	2.7 (-2.6, 8.1)	<0.017

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Damsgaard 2008 18492834 Scandinavia	Healthy	EPA+DHA + high LA	3.1 g/d (Marine oil) [E:D 1.64]	Placebo + high LA	0	2 mo	nd	17	99.6	16	90	3.5 (-9, 15.9)	
		EPA+DHA + low LA	3.1 g/d (Marine oil) [E:D 1.64]	Placebo + low LA	0	2 mo	nd	14	102.1	17	104.6	5.4 (-15.1, 25.9)	
Finnegan 2003 12663273 UK		EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	Placebo	0	6 mo	Pill count	31	132.05	30	140.15	11.7 (-3.2, 26.7)	nd
	Healthy	EPA+DHA	0.8 g/d (marine oil margarine)	Placebo	0	6 mo	Pill count	30	131.66	30	140.15	-2.3 (-11.0, 6.4)	nd
Grieger 2014 24454276 Australia	Healthy	EPA+DHA	0.8 g/d (fish diet)	Placebo	EPA: 0.017 g/d, DHA: 0.004 g/d (red meat diet)	8 wk	Weighed food records	43	123.55	37	127.41	11.6 (0.9, 22.3)	nd
Rasmussen 2006 16469978 Scandinavia, Australia	Healthy	EPA+DHA (MUFA diet)	EPA 3.6 g/d, 2.4 g/d DHA (Marine oil)	Placebo (MUFA diet)	0	3 mo	Dietary records (biomarker confirmation)	39	141	40	141	7.1 (-0.2, 14.3)	nd
	Healthy	EPA+DHA (SFA diet)	EPA 3.6 g/d, 2.4 g/d DHA (Marine oil)	Placebo (SFA diet)	0	3 mo		41	141	42	141	1.1 (-9.5, 11.7)	nd
Bosch 2012 22686415 Canada	At risk	EPA+DHA	EPA: 0.465 g/d, DHA: 0.375 g/d (Marine oil) [E:D 1.24]	Placebo	0	6 y	nd	6281	112	6255	112	0.6 (-1.6, 2.8)	nd
Brinton 2013 23835245 USA	At risk	EPA+DHA	4 g/d (Marine oil)	Placebo	0	3 mo	nd	225	82	226	84	-6.3 (-11.6, -1.0)	0.007
		EPA+DHA	2 g/d (Marine oil)	Placebo	0	3 mo	nd	233	82	226	84	-3.8 (-9, 1.4)	0.09

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Derosa 2009 19397392 Italy	At risk	EPA+DHA	EPA: 0.9 g/d, DHA: 1.5 g/d (marine oil)	Placebo	0	6 mo	Pill count	168	148.5	165	149.9	0.7 (-0.8, 2.2)	nd
Ebrahimi 2009 19593941 Iran	At risk	EPA+DHA	EPA: 0.18, DHA: 0.12 (marine oil)	Placebo	0	6 mo	nd	47	145.56	42	143.24	5.4 (-50.6, 61.4)	nd
Holman 2009 19002433 UK	At risk	EPA+DHA	2 g/d	Placebo	0	4 mo	Pill count	371	nd	361	nd	-1.2 (-11.1, 8.8)	0.82
Jones 2014 24829493 Canada	At risk	EPA+DHA (+ALA)	EPA: 0.1 g/d, DHA: 3.5 g/d, DPA: 1.4 g/d (canola+DHA)	(ALA)	0	4 wk	Assessed by coordinators	130	129.3	130	129.3	4.2 (-1.8, 10.3)	<0.05
Kastelein 2014 24528690 Europe	At risk	EPA+DHA	EPA: 2.20 g/d, DHA: 0.80 g/d	Placebo	0	12 wk	Pill count	99	90.3	98	78.2	15.2 (7.1, 23.2)	<0.001
		EPA+DHA	EPA: 1.65 g/d, DHA: 0.60 g/d	Placebo	0	12 wk	Pill count	97	81.0	98	78.2	9.2 (1.9, 16.6)	NS
		EPA+DHA	EPA: 1.10 g/d, DHA: 0.40 g/d	Placebo	0	12 wk	Pill count	99	77.3	98	78.2	12.5 (5.2, 19.8)	<0.01
Liu 2003 Sweden	At risk	EPA+DHA	EPA: 1.7 g/d, DHA: 1.1 g/d	Placebo	0	12 wk	Pill count	29	180.31	22	173.75	5.4 (-13.3, 24.1)	NS
		EPA+DHA + simvastatin	EPA: 1.7 g/d, DHA: 1.1 g/d	Placebo + simvastatin	0	12 wk	Pill count	19	173.36	18	172.20	5.0 (-17, 27.1)	NS
Lungershausen 1994 7852747 Australia	At risk	EPA+DHA	EPA: 1.9 g/d, DHA: 1.5 g/d (marine oil)	Placebo	0	6 wk	Pill count	42	155.98	42	155.98	6.6 (-7.4, 20.6)	0.359
Maki 2010 20451686 US	At risk	EPA+DHA (+simvastatin)	EPA: 1.86 g/d, DHA: 1.5 g/d	Placebo (+simvastatin)	0	8 wk	Pill count	122	89.2	132	92.3	3.4 (-0.03, 6.8)	0.052
Maki 2013 23998969 US	At risk	EPA+DHA	4 g/d total oil (free fatty acid oil) [nd]	Placebo	0	1.5 mo	Biomarker confirmation	207	93.6	211	91.7	-0.5 (-4.1, 3.1)	NS

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA	2 g/d total oil (free fatty acid oil) [nd]	Placebo	0	1.5 mo	Biomarker confirmation	209	92.3	211	91.7	3.2 (-0.4, 6.8)	<0.05
Oh, 2014, 25147070 Korea	At risk	EPA+DHA	4 g/d (Marine oil)	Placebo	0	2 mo	Pill count	44	110	42	111	1.0 (-13.2, 15.2)	
		EPA+DHA	2 g/d (Marine oil)	Placebo	0	2 mo	Pill count	43	109	42	111	6.0 (8.1, 20.1)	
		EPA+DHA	1 g/d (Marine oil)	Placebo	0	2 mo	Pill count	44	109	42	111	3.0 (11.1, 17.1)	
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	0.85 g/d (Marine oil)	Placebo	0	5 y	Self-reported	6239	131.8	6266	132.5	-0.4 (-1.8, 1.1)	0.63
Shidfar 2003 12847992 Iran	At risk	EPA+DHA	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo	0	2.5 mo	nd	16	159.6	19	167.4	−4 (−34.7, 26.7)	
		EPA+DHA +vitamin C	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo + vitamin C	0	2.5 mo	nd	16	150.8	17	160.6	10.3 (−18.8, 39.4)	
Sirtori 1997 9174486 Italy	At risk	EPA+DHA	2.57 g/d (Marine oil) [E:D 1.45]	Placebo	0	6 mo	nd	470	135.1	465	135.1	6.6 (6.3, 6.8)	
Tierney 2011 20938439 Europe	At risk	EPA+DHA	EPA 0.26 g/d, DHA 0.19 g/d (suppl) [E:D 1.5]	Placebo	0	3 mo	Pill count and plasma FA	100	127.80	106	122.39	−5.41 (−17.73, 6.91)	nd
Vecka 2012 23183517 Czech	At risk	EPA+DHA	2.58 g/d (Marine oil) [E:D 2.74]	Placebo	0	1.5 mo	nd	60	nd	60	nd	10.4 (nd)	<0.01
Eritsland 1996 8540453 Norway	CVD	EPA+DHA	3.4 g/d (Marine Oil)	Placebo	0	9 mo	nd	260	177.22	251	177.99	4.0 (-3.8, 11.8)	nd
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1192	102	1236	100	-0.8 (-4.0, 2.4)	NS

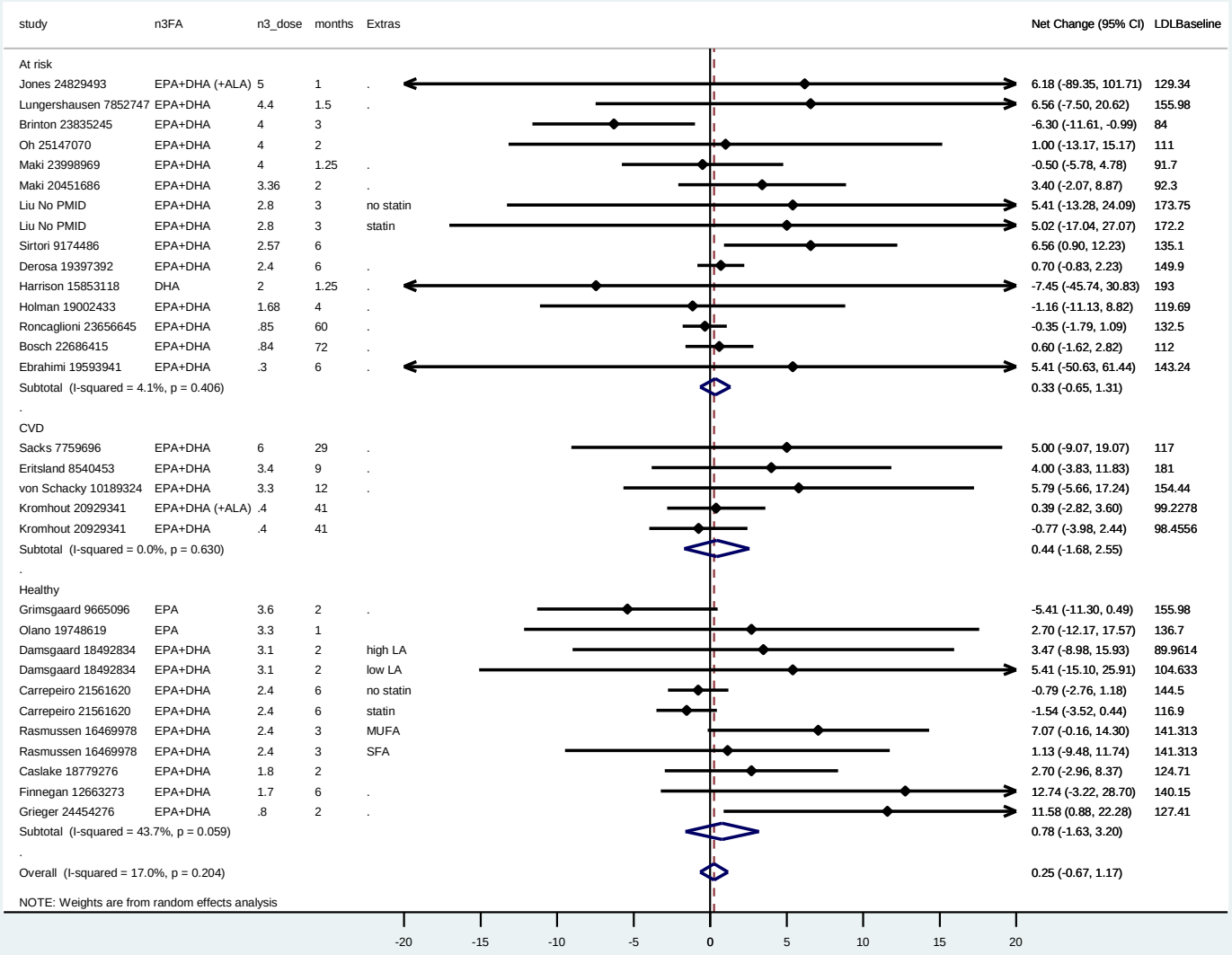
Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA (+ALA)	0.4 g/d (Marine oil) [E:D 3:2]	(ALA)	0			1212	98	1197	99	0.4 (-2.8, 3.6)	nd
Marchioli 2002 11997274 Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine Oil)	Placebo	0	42 mo	Measured at followup times	5666	136	5668	137	2 (nd)	nd
Rauch 2010 21060071 Germany	CVD	EPA+DHA	1 g/d (Marine oil) [E:D ratio 0.460:0.380]	Placebo	0	1 y	Pill count	1925	Not reported	1893	Not reported	0 (nd)	'Did not differ significantly between the study groups'
Sacks 1995 7759696 US	CVD	EPA+DHA	EPA: 2.88 g/d DHA: 3.12 g/d (Marine oil)	Placebo	0	2.4 y	Pill count (80% in INT, 90% in CONT)	31	122	28	117	5.0 (-9.1, 19.1)	nd
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	EPA: 0.386-0.401 g/d DHA: 0.464-0.481 g/d (Marine oil) [E:D 0.83]	Placebo	0	3.9 y	Measured at clinical exams, patient was compliant if drug administered for 80% of days. Both groups had ~30% compliance	3494	nd	3481	nd	"no differences"	nd
Von Schacky 1999 10189324 Canada	CVD	EPA+DHA	3.3 g/d	Placebo	0	12 mo	Pill count	112	158.30	111	154.44	5.8 (-5.7, 17.2)	NS
Marine oil vs Marine oil (doses)													

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	EPA+DHA	0.8 g/d (marine oil margarine)	6 mo	Pill count	31	132.05	30	131.66	14.0 (0.4, 27.7)	nd
Caslake 2008 18779276 UK	Healthy	EPA+DHA	1.8 g/d (Marine oil)	EPA+DHA	0.7 g/d (Marine oil)	2 mo	Pill count	312	148.5	312	148.5	0 (−6.3, 6.3)	NS
Brinton 2013 23835245 USA	At risk	EPA+DHA	4 g/d (Marine oil)	EPA+DHA	2 g/d (Marine oil)	3 mo	nd	225	82	233	82	−4	
Kastelein 2014 24528690 Europe	At risk	EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	3 mo	Pill count	99	90.3	97	81.0	5.9 (-2.6, 14.5)	nd
		EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	Pill count	99	90.3	99	77.3	2.7 (-5.9, 11.2)	nd
		EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	Pill count	97	81.0	99	77.3	-3.3 (-11.1, 4.6)	nd
Oh, 2014, 25147070 Korea	At risk	EPA+DHA	4 g/d (Marine oil)	EPA+DHA	2 g/d (Marine oil)	2 mo	Pill count	44	110	43	109	−5 (18.7, 8.8)	
		EPA+DHA	4 g/d (Marine oil)	EPA+DHA	1 g/d (Marine oil)	2 mo	Pill count	44	110	44	109	1 (−13.1, 15.1)	
		EPA+DHA	2 g/d (Marine oil)	EPA+DHA	1 g/d (Marine oil)	2 mo	Pill count	43	109	44	109	6 (−8.1, 20.1)	
Tatsuno 2013 24314359 Japan	At Risk	EPA+DHA	EPA: 1.86 g/d, DHA: 1.50 g/d (Marine oil)	EPA+DHA	EPA: 0.93 g/d, DHA: 0.75 g/d (Marine oil)	12 wk	Pill count	210	125.7	206	127.4	1.3 (−4.4, 7.0)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Maki 2013 23998969 US	At risk	EPA+DHA	4 g/d total oil (free fatty acid oil) [nd]	EPA+DHA	2 g/d total oil (free fatty acid oil) [nd]	1.5 mo	Biomarker confirmation	207	93.6	209	92.3	-3.7 (-7.3, -0.1)	
Marine oil vs Marine oil (miscellaneous)													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl)	DHA	3.6 g/d (suppl)	2 mo	pill count	72	156.8	77	156.8	-5.8 (-11.7, 0.1)	nd
Olano-Martin 2010 19748619 UK	Healthy	EPA	3.3 g/d (Marine oil)	DHA	3.7 g/d (Marine oil)	1 mo	nd	38	136.3	38	139.4	3.1 (-12.5, 18.7)	
Tatsuno 2013 24314359 Japan		EPA+DHA	EPA: 1.86 g/d, DHA: 1.50 g/d (Marine oil)	EPA	1.8 g/d	12 wk	Pill count	210	125.7	195	130.1	4.7 (−1.1, 10.5)	nd
		EPA+DHA	EPA: 0.93 g/d, DHA: 0.75 g/d (Marine oil)	EPA	1.8 g/d	12 wk	Pill count	206	127.4	195	130.1	3.4 (−2.6, 9.4)	nd
ALA vs Placebo													
Finnegan 2003 12663273 UK	Healthy	ALA	4.5 g/d (rapeseed oil margarine)	Placebo	0	6 mo	Pill count	30	137.07	30	140.15	-2.7 (-15.4, 10.0)	nd
Baxheinrich 2012 22894911 Germany	At risk	ALA	3.46 g/d (plant oil)	Placebo	ALA: 0.78 g/d	6 mo	Dietary records	40	132.05	41	134.75	1.9 (−12.0, 15.8)	0.181
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (canola)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	129.3	130	129.3	2.3 (-3.7, 8.4)	NS
		ALA	1.4 g/d (canolaOleic)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	129.3	130	129.3	0.4 (-5.7, 6.4)	NS

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Kromhout 2010 20929341 Netherlands	CVD	ALA	2 g/d (plant oil)	Placebo	0	40 mo	Audit of unused margarine tubs returned	1197	99	1236	100	0.4 (-2.8, 3.6)	NS
		ALA (+EPA+DHA)	2 g/d (plant oil)	(EPA+DHA)	0			1212	98	1192	102	1.5 (-1.7, 4.8)	nd
EPA+DHA vs ALA													
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	0.8 g/d (marine oil margarine)	ALA	4.5 g/d (rapeseed oil margarine)	6 mo	Pill count	30	131.66	30	137.07	14.5 (0.4, 28.6)	NS
		EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	ALA	4.5 g/d (ALA margarine)	6 mo	Pill count	31	132.05	30	137.07	0.4 (-10.8, 11.6)	NS
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	ALA	2 g/d (plant oil)	40 mo	Audit of unused margarine tubs returned	1192	102	1197	99	-1.2 (-4.4, 2.1)	nd
ALA vs ALA (doses)													
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (canola)	ALA	1.4 g/d (canolaOleic)	4 wk	Assessed by coordinators	130	129.34	130	129.34	1.9 (-4.1, 8.0)	.

Figure AD.2. Low density lipoprotein cholesterol: Randomized trials of marine oils



High Density Lipoprotein Cholesterol

Randomized Controlled Trials

Thirty-five RCTs provided data on effect of n-3 FA on high density lipoprotein cholesterol (HDL-c) (**Table AE.1**).[51, 56, 57, 63, 65, 66, 73, 77-79, 82, 90, 91, 97, 101, 110, 111, 114, 121, 122, 124, 126, 141, 145, 153-155, 159-161, 168, 169, 171, 176, 189]

Total n-3 FA vs. placebo

Two trials compared total n-3 FA (ALA+EPA+DHA) versus placebo, following 2708 patients for 1 and 40 months; one in people at increased risk for CVD,[110] one in people with CVD.[114] Baseline HDL-c measurements were 47 and 50 mg/dL. Compliance was measured in both studies, but not reported. The trial in an at risk population found a statistically significant increase in HDL-c with combined ALA 1.2 g/d (canola oil) and EPA+DHA+DPA 5 g/d (3.9 mg/dL; 95% CI 2.3, 5.4).[110] The trial in a CVD population found no significant effect on HDL-c with ALA 2 g/d and EPA+DHA 0.4 g/d.[114]

Marine oil vs. placebo

Thirty-three trials evaluated the effect of marine oils versus placebo on HDL-c.[56, 57, 63, 65, 66, 73, 77-79, 82, 90, 91, 97, 101, 110, 111, 114, 121, 122, 124, 126, 141, 145, 153, 155, 159-161, 168, 169, 171, 176, 189] Doses of EPA+DHA ranged from 0.3 to 6 g/d (median 2.4 g/d) and followup time ranged from 1 month to 6 years (median 3 months). Across populations, by meta-analysis, the summary net difference in HDL-c with EPA+DHA versus placebo (or equivalent) was a statistically significant, but small, 1.2 mg/dL (95% CI 0.6, 1.8) (**Figure AE.2**).

Healthy population

Eight of the trials of marine oils versus placebo were conducted in healthy populations, comprising data from 1184 individuals with mean baseline HDL-c ranging from 45 to 57.9 mg/dL and followup duration from 1 to 6 months.[63, 65, 66, 82, 90, 91, 97, 145] Two studies compared both EPA (3.3 and 3.8 g/d) and DHA (3.6 and 3.7 g/d), separately, to placebo;[91, 145] all other evaluated supplements with both EPA+DHA, with doses ranging from 0.7 to 6 g/d. Compliance was verified with pill counts, dietary records, or biomarker confirmation in six of the studies. One trial found significant net increases in HDL-c with marine oil (at two different doses, 0.7 and 1.8 g/d) of 2.3 mg/dL (95% CI 0.2, 4.5). One study, of DHA 3.8 g/d alone, found a significant net decrease in HDL-c (−5.4 mg/dL; 95% CI −6.7, −4.1), but not with EPA 3.6 g/d. The pooled effect size was a statistically significant, but small, 1.3 mg/dL (95% CI 0.2, 2.3).

At risk for CVD population

Nineteen of the trials were conducted in populations at increased risk of CVD, comprising data from 29,608 individuals with mean baseline HDL-c ranging from 28.7 to 65.6 mg/dL and followup duration from 1.5 months to 6 years.[56, 57, 73, 77, 78, 97, 101, 110, 111, 121, 122, 124, 153, 159-161, 169, 171, 189] One study compared DHA (2 g/d) to placebo;[97] all other evaluated supplements with both EPA+DHA, with doses ranging from 0.3 to 6 g/d. Compliance was verified with pill counts, dietary records, self-report or biomarker confirmation in 11 of the studies. Thirteen of the 17 trials found nonsignificant effects of EPA+DHA on HDL-c; net change HDL-c varied

between −5 and 9.3 mg/dL. The pooled effect size was a statistically significant, but small, 1.1 mg/dL (95% CI 0.2, 1.9).

CVD population

Seven of the trials were conducted in people with CVD, comprising data from 14,755 individuals with mean baseline HDL-c ranging from 39 to 50.2 mg/dL and followup duration from 9 months to 3.9 years.[79, 114, 126, 141, 155, 168, 176] Compliance was verified in four of the studies, by pill count or equivalent. Two of the seven trials found significant net increases in HDL-c, but net change HDL-c varied from −1.0 to 4.7 mg/dL. The pooled effect size was a statistically significant, but small, 1.5 mg/dL (95% CI 0.4, 2.7).

RCT subgroup analyses

Eight of the trials compared effects of marine oils in different subgroups of participants; three reported statin vs no statin,[101, 121, 171] one with or without vitamin C,[159] two men vs women,[65, 161] one older vs younger age,[65] and one impaired glucose tolerance versus normoglycemia.[160] One study found a larger effect of marine oil among participants who were also exercising (men 9.3 mg/dL; women 7.6 mg/dL) than in groups not exercising (men 1.7 mg/dL; women −0.9 mg/dL), although it was unclear whether these differences were significantly different from each other.[161] Another study found a small but significantly different effect ($P<0.05$) of marine oil 2.6 g/d in men with impaired glucose tolerance (0.8 mg/dL) than those with normoglycemia (0.4 mg/dL).[160]

By meta-regression, across studies there were no significant differences in effect (interactions) by HDL-c baseline ($P=0.87$), n-3 FA dose ($P=0.36$), followup duration ($P=0.43$), or population (at risk $P=0.64$; CVD $P=0.28$).

Marine oil, comparison of different doses

Six RCTs directly compared different doses of marine oils (EPA+DHA),[57, 65, 82, 111, 166, 167, 189] between 0.7 and 4 g/d. All comparisons were nonsignificant for effect on HDL-c, with estimates of differences ranging from −3.0 mg/dL (95% CI −6.4, 0.4; 2 vs. 1 g/d) to 1 mg/dL (2 studies; 3.4/4 vs. 1.7/2 g/d).

ALA vs. placebo

Four trials compared ALA versus placebo (or equivalent) in 661 people at increased risk of CVD and one trial of 4837 people with CVD.[51, 82, 110, 114] ALA doses ranged from 1.4 to 5.9 g/d and followup ranged from 1 to 40 months. All studies assessed compliance. Effect on HDL-c ranged from −1.5 to 0.8 mg/d, but all effects were statistically nonsignificant.

ALA, comparison of different doses

One trial compared ALA 5.9 and 1.4 g/d and found no difference in effect on HDL-c.[110]

Comparison of different specific n-3 FA

Two trials directly compared EPA (3.8 or 3.3 g/d) to DHA (3.6 or 3.7 g/d).[91, 145] Both found similar, nonsignificant effects on HDL-c with EPA or DHA. One trial compared two doses of EPA+DHA (3.4 and 1.7 g/d) to EPA 1.8 g/d,[166] with no differences between marine oil formulations. Two trials compared EPA+DHA to ALA. One compared two doses of EPA+DHA (1.7 and 0.8 g/d) to ALA 4.5 g/d;[82] both comparisons were nonsignificant with similar net

differences (1.5 and 2.3 mg/dL). The second trial compared EPA+DHA 0.2 g/d to ALA 2 g/d; the study did not report a significant difference, but a calculated net difference was statistically significant favoring EPA+DHA (net difference 1.9 mg/dL; 95% CI 0.9, 3.0).[114]

Observational Studies

Observational studies did not evaluate HDL-c.

Table AE.1. High density lipoprotein cholesterol: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Total n-3 FA vs. Placebo													
Jones 2014 24829493 Canada	At risk	ALA + EPA+DHA	ALA: 1.2 g/d, EPA: 0.1 g/d, DHA: 3.5 g/d, DPA: 1.4 g/d (canola+DHA)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	47.10	130	47.10	3.9 (2.3, 5.4)	<0.05
Kromhout 2010 20929341 Netherlands	CVD	ALA + EPA+DHA	0.4 g/d EPA+DHA; 2 g/d ALA (Marine oil, plant oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1212	50	1236	50	-0.4 (-1.5, 0.7)	NS
Marine oil vs Placebo													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl)	Placebo	0	2 mo	pill count	75	51.35	77	54.44	0.8 (-0.6, 2.2)	0.4
		DHA	3.6 g/d (suppl)	Placebo	0	2 mo	pill count	72	52.51	77	54.44	2.7 (1.2, 4.2)	0.0005
Olano-Martin 2010 19748619 UK	Healthy	EPA	3.3 g/d (Marine oil)	Placebo	0	1 mo	nd	38	136.3	38	136.7	-0.4 (-6.0, 5.3)	
		DHA	3.7 g/d (Marine oil)	Placebo	0	1 mo	nd	38	139.4	38	136.7	1.2 (-2.7, 5.0)	
Harrison 2004 15853118 Scotland	At risk	DHA	2 g/d (food fortification)	Placebo	0	1.25 mo	Food diary (biomarker confirmation)	101	63.7	112	65.6	-0.2 (-0.6, 0.3)	nd
Carrepeiro 2011 21561620 Brazil	Healthy	EPA+DHA + Statin	2.4 g/d (Marine oil)	Placebo + Statin	0	6 mo	nd	20	50.1	20	50.6	1.9 (nd)	

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA	2.4 g/d (Marine oil)	Placebo	0	6 mo	nd	23	52.4	23	49.6	-1.3 (nd)	
Caslake 2008 18779276 UK	Healthy	EPA+DHA	1.8 g/d (Marine oil)	Placebo	0	2 mo	Pill count	312	65.6	312	65.6	2.3 (0.2, 4.5)	<0.017
		EPA+DHA	0.7 g/d (Marine oil)	Placebo	0	2 mo	Pill count	312	65.2	312	65.6	2.3 (0.2, 4.5)	<0.017
Damsgaard 2008 18492834 Scandinavia	Healthy	EPA+DHA + high LA	3.1 g/d (Marine oil) [E:D 1.64]	Placebo + high LA	0	2 mo	nd	17	57.1	16	52.5	0.4 (-5.7, 6.4)	
		EPA+DHA + low LA	3.1 g/d (Marine oil) [E:D 1.64]	Placebo + low LA	0	2 mo	nd	14	57.9	17	57.9	3.1 (-7.8, 14)	
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	Placebo	0	6 mo	Pill count	31	51.74	30	52.12	1.4 (-2.1, 4.8)	nd
		EPA+DHA	0.8 g/d (marine oil margarine)	Placebo	0	6 mo	Pill count	30	52.90	30	52.12	2.8 (-0.2, 5.7)	nd
Grieger 2014 24454276 Australia	Healthy	EPA+DHA	0.8 g/d (fish diet)	Placebo	EPA: 0.017 g/d, DHA: 0.004 g/d (red meat diet)	8 wk	Weighed food records	43	65.64	37	61.776	0 (-10.7, 10.7)	nd
Sacks 1994 8021472 USA	Healthy	EPA+DHA	3g/d (Marine oil) [E:D 1.44:0.96]]	Placebo	0	6 mo	Pill count	84	46	84	45	1.8 (-0.9, 4.5)	ns
Bosch 2012 22686415 Canada	At risk	EPA+DHA	EPA: 0.465 g/d, DHA: 0.375 g/d (Marine oil) [E:D 1.24]	Placebo	0	6 y	nd	6281	46	6255	46	0.1 (-0.7, 0.9)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Brinton 2013 23835245 USA	At risk	EPA+DHA	4 g/d (Marine oil)	Placebo	0	3 mo	nd	226	37	227	39	-5.0 (-8.8, -1.2)	0.0013
		EPA+DHA	2 g/d (Marine oil)	Placebo	0	3 mo	nd	234	38	227	39	-2.3 (-5.6, 1.0)	0.1265
Derosa 2009 19397392 Italy	At risk	EPA+DHA	EPA: 0.9 g/d, DHA: 1.5 g/d (marine oil)	Placebo	0	6 mo	Pill count	168	38.4	165	39.7	3.9 (2.7, 5.1)	nd
Ebrahimi 2009 19593941 Iran	At risk	EPA+DHA	EPA: 0.18, DHA: 0.12 (marine oil)	Placebo	0	6 mo	nd	47	45.56	42	47.49	-0.4 (-13, 12.2)	nd
Einvik 2010 20389249 Norway	At risk	EPA+DHA	2.4g/d (Marine oil) [E:D 1.176:0.84]	Placebo	0	3 y	Pharmacy records/pill count	70	54.8	68	55.2	2.7 (-2.4, 7.9)	ns
		EPA+DHA + diet	2.4g/d (Marine oil) [E:D 1.176:0.84]	Placebo + diet	0	3 y	Pharmacy records/pill count	69	54.8	71	54.1	0.8 (-4.6, 6.2)	
Holman 2009 19002433 UK	At risk	EPA+DHA	2 g/d	Placebo	0	4 mo	Pill count	371	nd	361	nd	0.8 (-0.1, 1.6)	0.082
Jones 2014 24829493 Canada	At risk	EPA+DHA (+ALA)	EPA: 0.1 g/d, DHA: 3.5 g/d, DPA: 1.4 g/d	(ALA)	0	4 wk	Assessed by coordinators	130	47.10	130	47.10	3.9 (2.3, 5.4)	nd
Kastelein 2014 24528690 Europe	At risk	EPA+DHA	EPA: 2.20 g/d, DHA: 0.80 g/d	Placebo	0	12 wk	Pill count	99	28.7	98	28.7	1.1 (-0.5, 2.8)	NS
		EPA+DHA	EPA: 1.65 g/d, DHA: 0.60 g/d	Placebo	0	12 wk	Pill count	97	28.0	98	28.7	0.5 (-1.1, 2.2)	NS
		EPA+DHA	EPA: 1.10 g/d, DHA: 0.40 g/d	Placebo	0	12 wk	Pill count	99	27.3	98	28.7	1.5 (-0.2, 3.1)	NS

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Liu 2003 Sweden	At risk	EPA+DHA	EPA: 1.7 g/d, DHA: 1.1 g/d	Placebo	0	12 wk	Pill count	29	59.07	22	59.07	2.3 (-7.3, 12.0)	NS
		EPA+DHA + simvastatin	EPA: 1.7 g/d, DHA: 1.1 g/d	Placebo + simvastatin	0	12 wk	Pill count	19	55.21	18	64.09	2.3 (-9.3, 14.0)	NS
Lungershausen 1994 7852747 Australia	At risk	EPA+DHA	EPA: 1.9 g/d, DHA: 1.5 g/d (marine oil)	Placebo	0	6 wk	Pill count	42	39.8	42	39.8	0.8 (-2.7, 4.3)	0.664
Maki 2010 20451686 US	At risk	EPA+DHA (+simvastatin)	EPA: 1.86 g/d, DHA: 1.5 g/d	Placebo (+simvastatin)	0	8 wk	Pill count	122	47.3	132	44.7	2.5 (1.3, 3.7)	<0.001
Oh, 2014, 25147070 Korea	At risk	EPA+DHA	4 g/d (Marine oil)	Placebo	0	2 mo	Pill count	44	40	42	42	-1.0 (4.1, 2.2)	
		EPA+DHA	2 g/d (Marine oil)	Placebo	0	2 mo	Pill count	43	43	42	42	-2.0 (5.1, 1.2)	
		EPA+DHA	1 g/d (Marine oil)	Placebo	0	2 mo	Pill count	44	41	42	42	1.0 (2.4, 4.4)	
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	0.85 g/d (Marine oil)	Placebo	0	5 y	Self-reported	6239	50.9	6266	51.2	0.5 (0, 1.1)	0.04
Shidfar 2003 12847992 Iran	At risk	ALA + EPA+DHA	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo	0	2.5 mo	nd	16	39.1	19	39.2	-0.3 (-6.8, 6.2)	
		ALA + EPA+DHA + vitamin C	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo + vitamin C	0	2.5 mo	nd	16	53.3	17	37.2	-14.9 (-20.2, -9.6)	
Sirtori 1997 9174486 Italy	At risk	EPA+DHA	2.57 g/d (Marine oil) [E:D 1.45]	Placebo	0	6 mo	nd	470	39.8	465	39.8	0.4 (0.3, 0.5)	

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Soares 2014 24652053 Brazil	At risk (male)	EPA+DHA (+diet)	1 g/d (Marine oil) unspecified n-3 FA composition	Placebo	0	3 mo	Not reported	6	43.0	6	37.3	1.7 (-3.9, 7.3)	NS
	At risk (female)	EPA+DHA (+diet)						17	48.6	18	48.5	-0.9 (-2.9, 1.1)	NS
	At risk (male)	EPA+DHA (+diet/exercise)						4	36.0	6	34.8	9.3 (1.2, 17.4)	NS
	At risk (female)	EPA+DHA (+diet/exercise)						17	44.1	13	48.1	7.6 (5.4, 9.8)	NS
Tierney 2011 20938439 Europe	At risk	EPA+DHA	EPA 0.26 g/d, DHA 0.19 g/d (suppl) [E:D 1.5]	Placebo	0	3 mo	Pill count and plasma FA	100	42.86	106	42.08	0.77 (-2.439, 3.983)	nd
Vecka 2012 23183517 Czech	At risk	EPA+DHA	2.58 g/d (Marine oil) [E:D 2.74]	Placebo	0	1.5 mo	nd	60	nd	60	nd	1.9 (-25.4, 29.2) [difference of final values]	
Eritsland 1996 8540453 Norway	CVD	EPA+DHA	3.4 g/d (Marine Oil)	Placebo	0	9 mo	nd	260	40.93	251	38.6	2.0 (0, 4.0)	nd
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1192	50	1236	50	1.2 (0.1, 2.2)	NS
		EPA+DHA (+ALA)	0.4 g/d (Marine oil) [E:D 3:2]	(ALA)	0			1212	50	1197	50	0.4 (-0.7, 1.5)	nd
Marchioli 2002 11997274 Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine Oil)	Placebo	0	42 mo	Measured at followup times	5666	41	5668	41	0 (nd)	nd
Nilsen 2001 11451717 Norway	CVD	EPA+DHA	4 g/d (Marine oil) [E:D 1:2]	Placebo	0	Median 1.5 y	Unspecified method, but measured	119	--	120	--	4.7 (1.8, 7.7)	<0.001

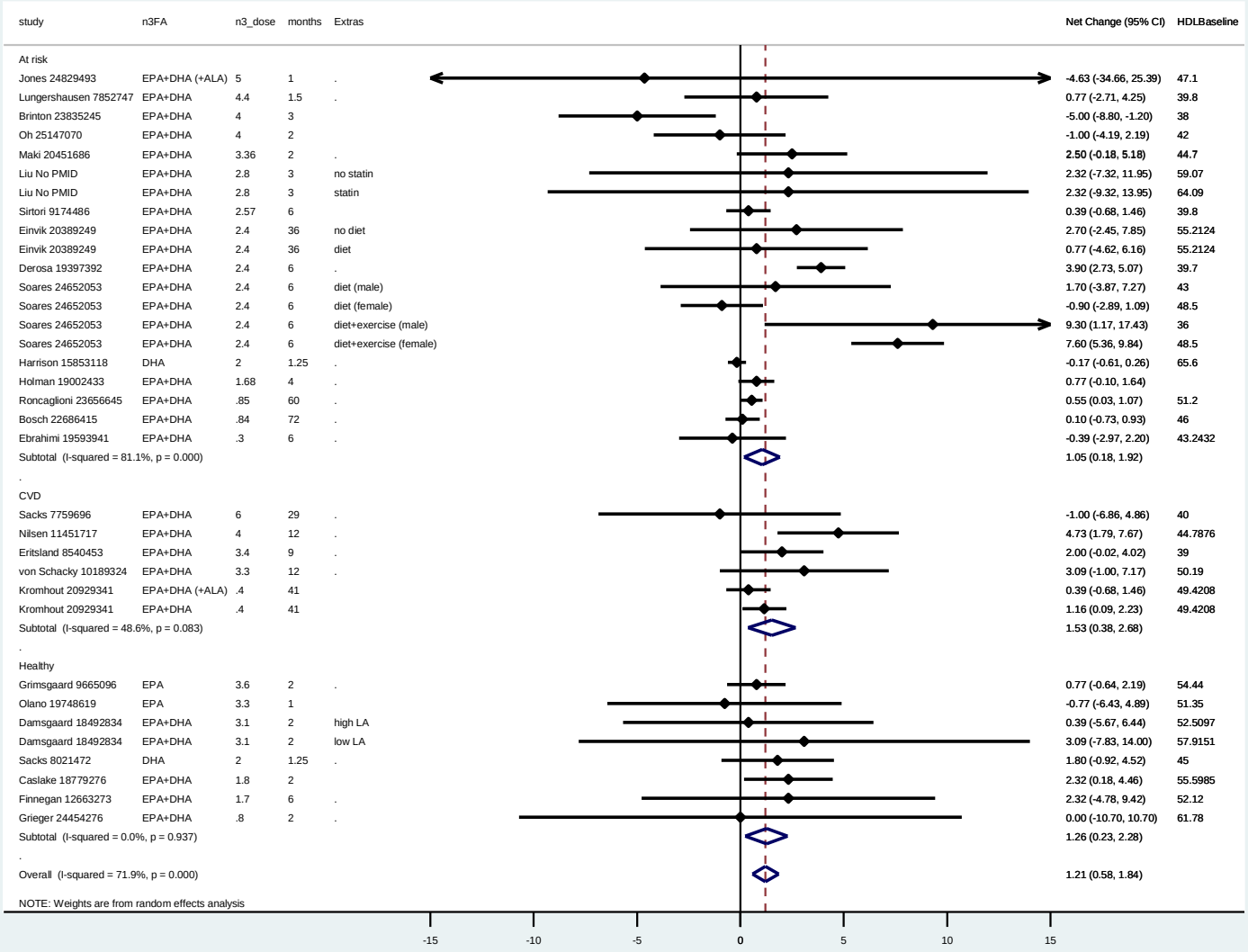
Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Sacks 1995 7759696 US	CVD	EPA+DHA	EPA: 2.88 g/d DHA: 3.12 g/d (Marine oil)	Placebo	0	2.4 y	Pill count (80% in INT, 90% in CONT)	31	41	28	40	-1.0 (-6.9, 4.9)	nd
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	EPA: 0.386-0.401 g/d DHA: 0.464-0.481 g/d (Marine oil) [E:D 0.83]	Placebo	0	3.9 y	Measured at clinical exams, patient was compliant if drug administered for 80% of days. Both groups had ~30% compliance	3494	nd	3481	nd	"no differences"	nd
Von Schacky 1999 10189324 Canada	CVD	EPA+DHA	3.3 g/d	Placebo	0	12 mo	Pill count	112	50.97	111	50.19	3.1 (-1.0, 7.2)	NS
Marine oil vs Marine oil (miscellaneous)													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl)	DHA	3.6 g/day (suppl)	2 mo	pill count	72	52.51	77	51.35	-1.9 (-3.5, -0.4)	0.009
Olano-Martin 2010 19748619 UK	Healthy	EPA	3.3 g/d (Marine oil)	DHA	3.7 g/d (Marine oil)	1 mo	nd	38	136.3	38	139.4	1.5 (-3.8, 6.9)	
Tatsuno 2013 24314359 Japan	At Risk	EPA+DHA	EPA: 0.93 g/d, DHA: 0.75 g/d (Marine oil)	EPA	1.8 g/d	12 wk	Pill count	206	45.8	195	45.6	0.3 (-1.7, 2.3)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA	EPA: 1.86 g/d, DHA: 1.50 g/d (Marine oil)	EPA	1.8 g/d	12 wk	Pill count	210	45.7	195	45.6	1.3 (-0.7, 3.3)	nd
Marine oil vs Marine oil (doses)													
Caslake 2008 18779276 UK	Healthy	EPA+DHA	1.8 g/d (Marine oil)	EPA+DHA	0.7 g/d (Marine oil)	2 mo	Pill count	312	65.6	312	65.2	0 (-2.5, 2.5)	NS
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	EPA+DHA	0.8 g/d (marine oil margarine)	6 mo	Pill count	31	51.74	30	52.90	-1.4 (-5.2, 2.5)	nd
Brinton 2013 23835245 USA	At risk	EPA+DHA	4 g/d (Marine oil)	EPA+DHA	2 g/d (Marine oil)	3 mo	nd	226	37	234	38	0 (nd)	
Kastelein 2014 24528690 Europe	At risk	EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	3 mo	Pill count	99	28.7	97	28.0	0.6 (-1.1, 2.3)	nd
		EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	Pill count	99	28.7	99	27.3	-0.4 (-2.0, 1.3)	nd
		EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	Pill count	97	28.0	99	27.3	-1.0 (-2.6, 0.7)	nd
Oh, 2014, 25147070 Korea	At risk	EPA+DHA	4 g/d (Marine oil)	EPA+DHA	2 g/d (Marine oil)	2 mo	Pill count	44	40	43	43	2 (1.3, 5.4)	
		EPA+DHA	4 g/d (Marine oil)	EPA+DHA	1 g/d (Marine oil)	2 mo	Pill count	44	40	44	41	0 (3.5, 3.5)	

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA	2 g/d (Marine oil)	EPA+DHA	1 g/d (Marine oil)	2 mo	Pill count	43	43	44	41	-3 (6.4, .4)	
Tatsuno 2013 24314359 Japan	At Risk	EPA+DHA	EPA: 0.93 g/d, DHA: 0.75 g/d (Marine oil)	EPA+DHA	EPA: 1.86 g/d, DHA: 1.50 g/d (Marine oil)	12 wk	Pill count	206	45.8	210	45.7	-1 (-3.034, 1.034)	nd
ALA vs Placebo													
Finnegan 2003 12663273 UK	Healthy	ALA	4.5 g/d (rapeseed oil margarine)	Placebo	0	6 mo	Pill count	30	49.81	30	52.12	0.5 (-3.1, 4.1)	nd
Baxheinrich 2012 22894911 Germany	At risk	ALA	3.46 g/d (plant oil)	Placebo	ALA: 0.78 g/d	6 mo	Dietary records	40	52.90	41	55.21	2.3 (-3.0, 7.6)	0.235
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (canola)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	47.10	130	47.10	0 (-1.5, 1.5)	NS
		ALA	1.4 g/d (canolaOleic)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	47.10	130	47.10	-0.8 (-2.3, 0.7)	NS
Kromhout 2010 20929341 Netherlands	CVD	ALA	2 g/d (plant oil)	Placebo	0	40 mo	Audit of unused margarine tubs returned	1197	49	1236	49	-0.8 (-1.8, 0.3)	NS
		ALA (+EPA+DHA)	2 g/d (plant oil)	(EPA+DHA)	0			1212	50	1192	50	-1.5 (-2.6, -0.5)	nd
ALA vs ALA (doses)													
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (canola)	ALA	1.4 g/d (canolaOleic)	4 wk	Assessed by coordinators	130	47.10	130	47.10	0.8 (-0.7, 2.3)	NS
Marine oil vs ALA													

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	ALA	4.5 g/d (rapeseed oil margarine)	6 mo	Pill count	31	51.74	30	49.81	0.9 (-2.8, 4.7)	nd
		EPA+DHA	0.8 g/d (marine oil margarine)	ALA	4.5 g/d (ALA margarine)	6 mo	Pill count	30	52.90	30	49.81	2.3 (-1.7, 6.3)	nd
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	ALA	2 g/d (plant oil)	40 mo	Audit of unused margarine tubs returned	1192	50	1197	49	1.9 (0.9, 3.0)	nd

Figure AE.2. High density lipoprotein cholesterol: Randomized trials of marine oils



Triglycerides

Randomized Controlled Trials

Thirty-seven RCTs provided data on effect of n-3 FA on triglycerides (Tg) (**Table AF.1**).[51, 56, 57, 63, 65, 66, 73, 76-79, 82, 90, 91, 101, 110, 111, 114, 121, 122, 124, 126, 141, 143, 145, 150, 153, 155, 159, 160, 166, 168, 169, 171, 176, 189]

Total n-3 FA vs. placebo

Two trials compared total n-3 FA (ALA+EPA+DHA) versus placebo, following 2708 patients for 1 and 40 months; one in people at increased risk for CVD,[110] one in people with CVD.[114] Baseline Tg measurements were 148 and 150 mg/dL. Compliance was measured in both studies, but not reported. The trial in an at risk population found a statistically significant decrease in Tg with combined ALA 1.2 g/d (canola oil) and EPA+DHA+DPA 5 g/d (−27 mg/dL; 95% CI −45, −10).[110] The trial in a CVD population found no significant effect on Tg with ALA 2 g/d and EPA+DHA 0.4 g/d.[114]

Marine oil vs. placebo

Thirty-four trials evaluated the effect of marine oils versus placebo on Tg.[56, 57, 63, 65, 66, 73, 76-79, 82, 90, 91, 101, 110, 111, 114, 121, 122, 124, 126, 141, 143, 145, 150, 153, 155, 159, 160, 168, 169, 171, 176, 189] Doses of EPA+DHA±DPA ranged from 0.3 to 6 g/d (median 2.4 g/d) and followup time ranged from 1 month to 6 years (median 3 months). All but two studies found net decreases in Tg with EPA+DHA. Across populations, the summary net difference in Tg with EPA+DHA versus placebo (or equivalent) was a statistically significant −23 mg/dL (95% CI −29, −18) among studies reporting sufficient data to be included in meta-analysis. As will be described below, net change Tg varied across studies by mean baseline Tg and, possibly, by EPA+DHA dose, but did not vary significantly by population (**Figure AF.2**).

Healthy population

Seven of the trials were conducted in 1181 generally healthy participants.[63, 65, 66, 82, 90, 91, 145] Two of the trials evaluated both purified EPA and DHA separately (3.3 to 3.8 g/d); the rest evaluated EPA+DHA (0.8 to 3.1 g/d). Followup ranged from 1 to 6 months. Four studies evaluated compliance with pill count or weighed food records. Baseline Tg ranged from 80 to 150 mg/dL. Net difference between marine oil and placebo varied widely across studies from −42 to 6 mg/dL. The pooled effect size was a significant −8 mg/dL (95% CI −12, −3).

At risk for CVD population

Eighteen trials compared EPA+DHA to placebo (or equivalent) in 28,817 people at increased risk of CVD.[56, 57, 73, 76-78, 101, 110, 111, 121, 122, 124, 153, 160, 171, 189] EPA+DHA dosages ranged from 0.3 to 5 g/d and followup ranged from 1 month to 6 years. Eleven of the studies measured compliance by pill count, coordinator "assessment," or self-report. Mean baseline Tg ranged from 111 to 315 mg/d in 13 of the trial, was 682 mg/d in one study that included only people with severe hypertriglyceridemia (≥500 mg/d),[111] and was not reported in two trials. Excluding the trial of severe hypertriglyceridemia, net change Tg with EPA+DHA ranged from −82 (difference between final values) to −7 mg/dL. The study of people with hypertriglyceridemia found large, significant net reductions of Tg with EPA+DHA doses of 1.5, 2.25, and 3 g/d of

−156 mg/d (lower two doses) and −173 mg/d (3 g/d). The pooled effect size (with the hypertriglyceridemia study) was a significant −38 mg/dL (95% CI −50, −26); without Kastelein, the pooled net difference was similar: −33 mg/dL (95% CI −45, 22).

CVD population

Nine trials compared EPA+DHA to placebo in 28,831 people with CVD.[79, 114, 126, 141, 143, 150, 155, 168, 176] EPA+DHA dosages ranged from 0.4 to 6 g/d and followup ranged from 9 months to 3.9 y. All but one study measured, but few reported, compliance. Mean baseline Tg ranged from 137 to 191 mg/d when reported. Across trials, net change Tg with EPA+DHA ranged from −3 to −50 mg/dL. The pooled effect size was a significant −20 mg/dL (95% CI −34, −7).

RCT subgroup analyses

The four studies that examined subgroup effects of EDA+DHA on Tg based on statin use all found no significant interaction between marine oil and statins (Carrepeiro 2011, Holman 2009, Liu 2003, Vecka 2012).[63, 101, 121, 171] In one study each, no significant differences in effect were seen in those on high or low linoleic acid diets (Damsgaard 2008),[66] in those receiving or not general diet counseling (Einvik 2010),[78] or in older or younger age groups (Caslake 2008).[65] One study found a significantly larger effect in people also taking a multivitamin (−76 mg/dL) than in those without the multivitamin (−28 mg/dL; P interaction <0.05), but Tg increased in only the group taking multivitamins and placebo (Earnest 2012).[76] In contrast, one found a net increase in Tg concentration in people also taking vitamin C (15 mg/dL, due to a smaller decrease in Tg concentration than in the vitamin C alone group) and a large net decrease in people not taking vitamin C (−109 mg/dL), but this difference in effect was not reported to be significantly different.[159] One study examined gender effect and found that men on higher dose EPA+DHA (1.8 g/d) had a larger effect than women (P<0.038; difference not reported), but similar effects at lower dose (0.7 g/d) (Caslake 2008).[65] One study found no difference in effect of EPA between people with either impaired glucose tolerance or noninsulin dependent diabetes or normoglycemia (Sirtori 1997), but among those with diabetes, those with lower HDL-c (≤35 mg/dL) had a greater effect of EPA+DHA on Tg (−23.3%) than those with higher HDL-c (−16.9%; P interaction <0.05).[160] This difference in effect by HDL-c levels, however, was not seen among those with normoglycemia. One study of people with diabetes (Brinton 2013) found that with higher dose EPA+DHA (4 g/d) there was no difference in change in Tg by hemoglobin A1c level, but at 2 g/d, those with higher A1c levels (>6.8%) had a smaller nonsignificant effect (−5% net change) than those with lower A1c levels (−15%, P<0.01), although the study did not analyze whether the interaction was significant.[57]

By meta-regression, across studies there were no significant differences in effect (interactions) by population (at risk P=0.35; CVD P=0.73) or followup duration (P=0.62). However, both mean baseline Tg level and EPA+DHA dose across studies were significantly associated with net change Tg. The primary metaregression was conducted excluding an outlier study (Kastelein 2014) of people with severe hypertriglyceridemia (Tg >500 mg/dL at baseline), who were found to have large net changes with EPA+DHA 3, 2.25, and 1.5 g/d.[111] Analyses with this study, however, yielded similar results. Controlling for both variables, each increase in mean baseline Tg level by 1 mg/dL was associated with a greater net change Tg of −0.12 mg/dL (95% CI −0.22, −0.03; P=0.013) (**Figure AF.3**). Each increase of EPA+DHA dose by 1 g/d was also associated with a greater net change Tg of −6.8 mg/dL (95% CI −11.4, −2.2; P=0.005) (**Figure**

AF.4). By spline analysis of the meta-regression, there was no clear inflection point where the association between dose and net change Tg substantially changed.

Marine oil, comparison of different doses

Six RCTs directly compared different doses of marine oils (EPA+DHA),[57, 65, 82, 111, 166, 189] between 0.7 and 4 g/d. The trials compared EPA+DHA doses between 0.7 and 4 g/d. Only one of the six trials found a significant difference between higher (3.4 g/d) and lower (1/7 g/d) EPA+DHA.[Tatsuno 2013] Although, most trials found no significant difference, the differences in effect on Tg between doses ranged from −39 to 6 mg/dL. A possible pattern could be discerned such that higher doses of 3.4 or 4 g/d reduced Tg by at least 30 mg/dL more than lower doses of 1 to 2 g/d (Brinton 2013: 4 vs. 2 g/d; Oh 2014: 4 vs 2 g/d and 2 vs. 1 g/d; Tatsuno 2013: 3.4 vs. 1.7 g/d). Higher doses ≤3 g/d (1.7-3 g/d) yielded much smaller relative differences in Tg change compared to lower doses (0.7-2.25 g/d) (−17 to 6 mg/dL) (Caslake 2008: 1.8 vs. 0.7 g/d; Finnegan 2003: 1/7 vs. 0.8 g/d; Kastelein 2014: 3 vs. 2.25 g/d, 3 vs. 1.5 g/d, and 2.25 vs. 1.5 g/d; Oh 2014: 2 vs. 1 g/d).

ALA vs. placebo

Four trials compared ALA supplementation versus placebo (or equivalent), following 5368 patients for 1 to 40 months; one in healthy people,[82] two in people at increased risk for CVD,[51, 110] and one in people with CVD.[114] Doses of ALA ranged from 1.4 to 5.9 g/d, and baseline Tg measurements ranged from 146 to 172 mg/dL. Compliance was measured in all studies, but not reported. All trials found no significant effect of total n-3 FA supplementation on Tg; the estimates of the net differences ranged from −22 to 23, mostly with wide confidence intervals.

Comparison of different specific n-3 FA

Two trials directly compared EPA (3.8 or 3.3 g/d) to DHA (3.6 or 3.7 g/d).[91, 145] Neither found a significant difference in effect on Tg between EPA and DHA. One trial compared two doses of EPA+DHA (3.4 and 1.7 g/d) to EPA 1.8 g/d,[166] finding significantly larger net reductions in Tg with either dose of EPA+DHA than EPA alone. Two trials compared EPA+DHA to ALA, one comparing two doses of EPA+DHA (1.7 and 0.8 g/d) to ALA 4.5 g/d,[82] one comparing 0.4 g/d EPA+DHA to 2 g/d ALA.[114] A possible dose effect of EPA+DHA was found in that the comparison with the highest dose of EPA+DHA (1.7 g/d) found a significantly greater effect of EPA+DHA than ALA (−28 mg/dL; 95% CI −49, −7) (Finnegan 2003), while in the same study a lower dose (0.8 g/d) had a smaller nonsignificant difference (−14 mg/dL), and the other study (Kromhout 2010), with EPA+DHA 0.4 g/d had no differential effect (2.7 mg/dL)

Observational Studies

Observational studies did not evaluate Tg.

Table AF.1. Triglycerides: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Total n-3 FA vs Placebo													
Jones 2014 24829493 Canada	At risk	ALA + EPA+DHA	ALA: 1.2 g/d, EPA: 0.1 g/d, DHA: 3.5 g/d, DPA: 1.4 g/d (canola+DHA)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	147.79	130	147.79	-27.4 (-44.8, -10.1)	<0.05
Kromhout 2010 20929341 Netherlands	CVD	ALA + EPA+DHA	0.4 g/d EPA+DHA; 2 g/d ALA (Marine oil, plant oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1212	145	1236	150	-8 (-16.6, 0.7)	
Marine oil vs Placebo													
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl)	Placebo	0	2 mo	pill count	75	108.85	77	107.96	-23 (-33.5, -12.6)	0.0001
		DHA	3.6 g/d (suppl)	Placebo	0	2 mo	pill count	72	109.73	77	107.96	-29.2 (-38.4, -20.0)	0.0001
Olano-Martin 2010 19748619 UK	Healthy	EPA	3.3 g/d (Marine oil)	Placebo	0	1 mo	nd	38	143.4	38	123	-41.6 (-69.9, -13.3)	
		DHA	3.7 g/d (Marine oil)	Placebo	0	1 mo	nd	38	132.7	38	123	-27.4 (-45.3, -9.5)	
Carrepeiro 2011 21561620 Brazil	Healthy	EPA+DHA	2.4 g/d (Marine oil)	Placebo	0	6 mo	nd	23	101.2	23	112.9	-1.8 (-3.8, 0.2)	0.077
		EPA+DHA + Statin	2.4 g/d (Marine oil)	Placebo + Statin	0	6 mo	nd	20	140.1	20	120.8	-2.0 (-4.0, 0)	0.054
Caslake 2008 18779276 UK	Healthy	EPA+DHA	1.8 g/d (Marine oil)	Placebo	0	2 mo	Pill count	312	113.3	312	112.4	-1.4 (-10.8, 7.9)	<0.017
		EPA+DHA	0.7 g/d (Marine oil)	Placebo	0	2 mo	Pill count	312	110.6	312	112.4	-8.0 (-17.3, 1.3)	<0.017
Damsgaard 2008 18492834 Scandinavia	Healthy	EPA+DHA + high LA	3.1 g/d (Marine oil) [E:D 1.64]	Placebo + high LA	0	2 mo	nd	17	71.7	16	79.6	-7.3 (-14.3, -0.4)	

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA + low LA	3.1 g/d (Marine oil) [E:D 1.64]	Placebo + low LA	0	2 mo	nd	14	113.3	17	89.4	-18.1 (-27.8, -8.5)	
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	Placebo	0	6 mo	Pill count	31	141.59	30	149.56	-5.7 (-24.0, 12.7)	nd
		EPA+DHA	0.8 g/d (marine oil margarine)	Placebo	0	6 mo	Pill count	30	146.02	30	149.56	7.7 (-3.6, 19.0)	nd
Grieger 2014 24454276 Australia	Healthy	EPA+DHA	0.8 g/d (fish diet)	Placebo	EPA: 0.017 g/d, DHA: 0.004 g/d (red meat diet)	8 wk	Weighed food records	43	97.35	37	123.89	0 (-24.5, 24.5)	nd
Bosch 2012 22686415 Canada	At risk	EPA+DHA	EPA: 0.465 g/d, DHA: 0.375 g/d (Marine oil) [E:D 1.24]	Placebo	0	6 y	nd	6281	142	6255	140	-14.5 (-22.8, -6.2)	<0.001
Brinton 2013 23835245 USA	At risk	EPA+DHA	4 g/d (Marine oil)	Placebo	0	3 mo	nd	226	264.8	227	259	-23.2 (-34.9, -11.5)	<0.0001
		EPA+DHA	2 g/d (Marine oil)	Placebo	0	3 mo	nd	234	254	227	259	-9.8 (-17.3, -2.3)	0.0005
Derosa 2009 19397392 Italy	At risk	EPA+DHA	EPA: 0.9 g/d, DHA: 1.5 g/d (marine oil)	Placebo	0	6 mo	Pill count	168	182.6	165	189.3	-59.2 (-67.4, -51.0)	nd
Earnest 2012 22811376 US	At risk	EPA+DHA	2 g/d EPA+DHA (Marine oil) [E:D ratio 0.76:0.44]	Placebo	0	3 mo	Pill count	21	111	23	111	-27.7 (-51.4, -4.0)	
		EPA+DHA + multivitamin	2 g/d EPA+DHA + (Marine oil) [E:D ratio 0.76:0.44]	Placebo + multivitamin	0	3 mo	Pill count	25	116	23	113	-75.7 (-98.5, -52.9)	
Ebrahimi 2009 19593941 Iran	At risk	EPA+DHA	EPA: 0.18, DHA: 0.12 (marine oil)	Placebo	0	6 mo	nd	47	155.75	42	145.13	-7.1 (nd)	nd
Einvik 2010 20389249 Norway	At risk	EPA+DHA	2.4 g/d EPA+DHA (Marine oil) [E:D ratio 0.66:1.1]	Placebo	0	3 y	Pharmacy records/pill count	70	152	68	150	-15.0 (-41.1, 11.1)	--

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA + diet	2.4 g/d EPA+DHA (Marine oil) [E:D ratio 0.66:1.1]	Placebo + diet	0	3 y	Pharmacy records/pill count	71	152	69	150	-20.4 (-44.3, 3.6)	
Holman 2009 19002433 UK	At risk	EPA+DHA	2 g/d	Placebo	0	4 mo	Pill count	371	nd	361	nd	-8.0 (-13.2, -2.7)	0.003
Jones 2014 24829493 Canada	At risk	EPA+DHA (+ALA)	EPA: 0.1 g/d, DHA: 3.5 g/d, DPA: 1.4 g/d (canola+DHA)	(ALA)	0	4 wk	Assessed by coordinators	130	147.79	130	147.79	-31 (-48.3, -13.6)	<0.05
Liu 2003 Sweden	At risk	EPA+DHA	EPA: 1.7 g/d, DHA: 1.1 g/d	Placebo	0	12 wk	Pill count	29	146.90	22	142.48	-39.8 (-76.4, -3.3)	<0.05
		EPA+DHA + simvastatin	EPA: 1.7 g/d, DHA: 1.1 g/d	Placebo + simvastatin	0	12 wk	Pill count	19	154.87	18	136.28	-35.4 (-79.6, 8.8)	<0.05
Lungershausen 1994 7852747 Australia	At risk	EPA+DHA	EPA: 1.9 g/d, DHA: 1.5 g/d (marine oil)	Placebo	0	6 wk	Pill count	42	141.59	42	141.59	-28.3 (-54.8, -1.8)	0.05
Kastelein 2014 24528690 Europe	At risk	EPA+DHA	EPA: 2.20 g/d, DHA: 0.80 g/d	Placebo	0	12 wk	Pill count	99	655	98	682	-173.1 (-250.3, -95.8)	<0.001
		EPA+DHA	EPA: 1.65 g/d, DHA: 0.60 g/d	Placebo	0	12 wk	Pill count	97	728	98	682	-156.3 (-238.8, -73.8)	<0.01
		EPA+DHA	EPA: 1.10 g/d, DHA: 0.40 g/d	Placebo	0	12 wk	Pill count	99	717	98	682	-156.4 (-238.1, -74.6)	<0.01
Maki 2010 20451686 US	At risk	EPA+DHA (+simvastatin)	EPA: 1.86 g/d, DHA: 1.5 g/d	Placebo (+simvastatin)	0	8 wk	Pill count	122	282	132	286.7	-68.8 (-83.7, -53.9)	<0.001
Oh, 2014, 25147070 Korea	At risk	EPA+DHA	4 g/d (Marine oil)	Placebo	0	2 mo	Pill count	44	287	42	281	-62.0 (-102.5, -21.5)	
		EPA+DHA	2 g/d (Marine oil)	Placebo	0	2 mo	Pill count	43	267	42	281	-30.0 (-73.1, 13.1)	
		EPA+DHA	1 g/d (Marine oil)	Placebo	0	2 mo	Pill count	44	286	42	281	-23.0 (-60.6, 14.6)	
Roncaglioni 2013 23656645 Italy	At risk	EPA+DHA	0.85 g/d (Marine oil)	Placebo	0	5 y	Self-reported	6239	150	6266	150	-8.1 (-11.4, -4.7)	<0.0001

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Shidfar 2003 12847992 Iran	At risk	EPA+DHA	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo	0	2.5 mo	nd	16	304	19	311.5	-109 (-177, -41)	
		EPA+DHA + vitamin C	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo + vitamin C	0	2.5 mo	nd	16	297.3	17	315	15.2 (-43.9, 74.3)	
Sirtori 1997 9174486 Italy	At risk	EPA+DHA	2.57 g/d (Marine oil) [E:D 1.45]	Placebo	0	6 mo	nd	470	293.8	465	297.3	-37.2 (-51.0, -23.3)	
Tierney 2011 20938439 Europe	At risk	EPA+DHA	EPA 0.26 g/d, DHA 0.19 g/d (suppl) [E:D 1.5]	Placebo	0	3 mo	Pill count and plasma FA	100	148.67	106	147.79	-19.47 (-44.664, 5.726)	nd
Vecka 2012 23183517 Czech	At risk	EPA+DHA	2.58 g/d (Marine oil) [E:D 2.74]	Placebo	0	1.5 mo	nd	60	nd	60	nd	-82.3 [difference of final values]	<0.001
Eritsland 1996 8540453 Norway	CVD	EPA+DHA	3.4 g/d (Marine oil)	Placebo	0	9 mo	nd	260	171.62	251	184.96	-32.0 (-49.6, -14.4)	
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	Placebo	0	40 mo	Audit of unused margarine tubs returned	1192	144	1236	150	-2.7 (-13.8, 8.5)	
		EPA+DHA (+ALA)	0.4 g/d (Marine oil) [E:D 3:2]	(ALA)	0			1212	145	1197	146	-2.7 (-11.3, 6.0)	
Marchioli 2002 11997274 Italy	CVD	EPA+DHA	0.850-0.882 g/d (Marine Oil)	Placebo	0	42 mo	Measured at followup times	5666	162	5668	162	-10 (nd)	
Nilsen 2001 11451717 Norway	CVD	EPA+DHA	4 g/d (Marine oil) [E:D 1:2]	Placebo	0	Median 1.5 y	Unspecified method, but measured	120	--	121	--	-36.9 (-55.4, -18.4)	Not reported
Nodari 2011 21215550 Italy	CVD	EPA+DHA	2 g/d EPA+DHA (Marine oil) [E:D ratio 0.9:1.5]	Placebo	0	1 y	Pill count	67	149	66	154	-7.0 (-29.0, 15.0)	--
Rauch 2010 21060071 Germany	CVD	EPA+DHA	1 g/d (Marine oil) [E:D ratio 0.460:0.380]	Placebo	0	1 y	Pill count	1925	Not reported	1893	Not reported	-5 (nd)	<0.01

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Sacks 1995 7759696 US	CVD	EPA+DHA	EPA: 2.88 g/d DHA: 3.12 g/d (Marine oil)	Placebo	0	2.4 y	Pill count (80% in INT, 90% in CONT)	31	128	28	137	-33.0 (-66.6, 0.6)	
Tavazzi 2008 18757090 Italy	CVD	EPA+DHA	EPA: 0.386-0.401 g/d DHA: 0.464-0.481 g/d (Marine oil) [E:D 0.83]	Placebo	0	3.9 y	Measured at clinical exams, patient was compliant if drug administered for 80% of days. Both groups had ~30% compliance	3494	1.42(median)	3481	nd	nd	<0.0001
Von Schacky 1999 10189324 Canada	CVD	EPA+DHA	3.3 g/d	Placebo	0	12 mo	Pill count	112	194.69	111	191.15	-49.6 (-81.5, -17.6)	<0.01
Marine oil vs Marine oil (dose)													
Caslake 2008 18779276 UK	Healthy	EPA+DHA	1.8 g/d (Marine oil)	EPA+DHA	0.7 g/d (Marine oil)	2 mo	Pill count	312	113.3	312	110.6	6.2 (-2.6, 15.0)	NS
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	6 mo	0.8 g/d (marine oil margarine)	EPA+DHA	Pill count	31	141.59	30	146.02	-13.4 (-30.8, 4.0)	nd
Brinton 2013 23835245 USA	At risk	EPA+DHA	4 g/d (Marine oil)	EPA+DHA	2 g/d (Marine oil)	3 mo	nd	226	264.8	234	254	-32.1 (nd)	
Kastelein 2014 24528690 Europe	At risk	EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	3 mo	nd	99	655	99	728	-16.8 (-86.1, 52.6)	nd

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
		EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	nd	99	655	99	717	-16.7 (-85.1, 51.8)	nd
		EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	nd	97	728	99	717	0.1 (-74.3, 74.4)	nd
Oh, 2014, 25147070 Korea	At risk	EPA+DHA	4 g/d (Marine oil)	EPA+DHA	2 g/d (Marine oil)	2 mo	Pill count	44	287	43	267	-32 (-77.2, 13.2)	
		EPA+DHA	4 g/d (Marine oil)	EPA+DHA	1 g/d (Marine oil)	2 mo	Pill count	44	287	44	286	-39 (-79.1, 1.1)	
		EPA+DHA	2 g/d (Marine oil)	EPA+DHA	1 g/d (Marine oil)	2 mo	Pill count	43	267	44	286	-7.0 (-49.7, 35.7)	
Tatsuno 2013 24314359 Japan	At Risk	EPA+DHA	EPA: 1.86 g/d, DHA: 1.50 g/d (Marine oil)	EPA+DHA	EPA: 0.93 g/d, DHA: 0.75 g/d (Marine oil)	12 wk	Pill count	210	277.5	206	296	-33.3 (-50.4, -16.2)	nd
Marine oil vs Marine oil (miscellaneous)													
Olano-Martin 2010 19748619 UK	Healthy	EPA	3.3 g/d (Marine oil)	DHA	3.7 g/d (Marine oil)	1 mo	nd	38	143.4	38	132.7	14.2 (-14.1, 42.5)	
Grimsgaard 1998 9665096 Norway	Healthy	EPA	3.8 g/d (suppl)	DHA	3.6 g/d (suppl)	2 mo	pill count	77	108.85	72	109.73	6.2 (-4.0, 16.4)	0.14
Tatsuno 2013 24314359 Japan	At Risk	EPA+DHA	EPA: 1.86 g/d, DHA: 1.50 g/d (Marine oil)	EPA	1.8 g/d	12 wk	Pill count	210	277.5	195	271.8	-35 (-53.348, -16.652)	nd
		EPA+DHA	EPA: 0.93 g/d, DHA: 0.75 g/d (Marine oil)	EPA	1.8 g/d	12 wk	Pill count	206	296	195	271.8	-24.8 (-42.22, -7.38)	nd
ALA vs Placebo													
Finnegan 2003 12663273 UK	Healthy	ALA	4.5 g/d (rapeseed oil margarine)	Placebo	0	6 mo	Pill count	30	146.90	30	149.56	22.0 (2.1, 41.9)	NS

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL (95% CI)	Reported P value
Baxheinrich 2012 22894911 Germany	At risk	ALA	3.46 g/d (plant oil)	Placebo	ALA: 0.78 g/d	6 mo	Dietary records	40	171.68	41	145.13	-22.1 (-59.0, 14.8)	0.020
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (canola)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	147.79	130	147.79	3.5 (-13.8, 20.9)	NS
			1.4 g/d (canolaOleic)	Placebo	0.2 g/d (CornSaff)	4 wk	Assessed by coordinators	130	147.79	130	147.79	7.1 (-10.3, 24.4)	NS
Kromhout 2010 20929341 Netherlands	CVD	ALA	2 g/d (plant oil)	Placebo	0	40 mo	Audit of unused margarine tubs returned	1197	146	1236	150	-5.3 (-15.1, 4.5)	
		ALA (+EPA+DHA)	2 g/d (plant oil)	(EPA+DHA)	0			1212	145	1192	144	-5.3 (-15.4, 4.8)	
ALA vs ALA (doses)													
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (canola)	ALA	1.4 g/d (canolaOleic)	4 wk	Assessed by coordinators	130	147.8	130	147.8	-3.5 (-13.8, 20.9)	NS
EPA+DHA vs ALA													
Finnegan 2003 12663273 UK	Healthy	EPA+DHA	1.7 g/d (marine oil capsule and marine oil margarine)	ALA	4.5 g/d (rapeseed oil margarine)	6 mo	Pill count	31	141.59	30	146.90	-27.7 (-48.7, -6.6)	nd
		EPA+DHA	0.8 g/d (marine oil margarine)	ALA	4.5 g/d (ALA margarine)	6 mo	Pill count	30	146.02	30	146.90	-14.3 (-33.3, 4.8)	nd
Kromhout 2010 20929341 Netherlands	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 3:2]	ALA	2 g/d (plant oil)	40 mo	Audit of unused margarine tubs returned	1192	144	1197	146	2.7 (-8.5, 13.8)	

Figure AF.2. Triglycerides: Randomized trials of marine oils

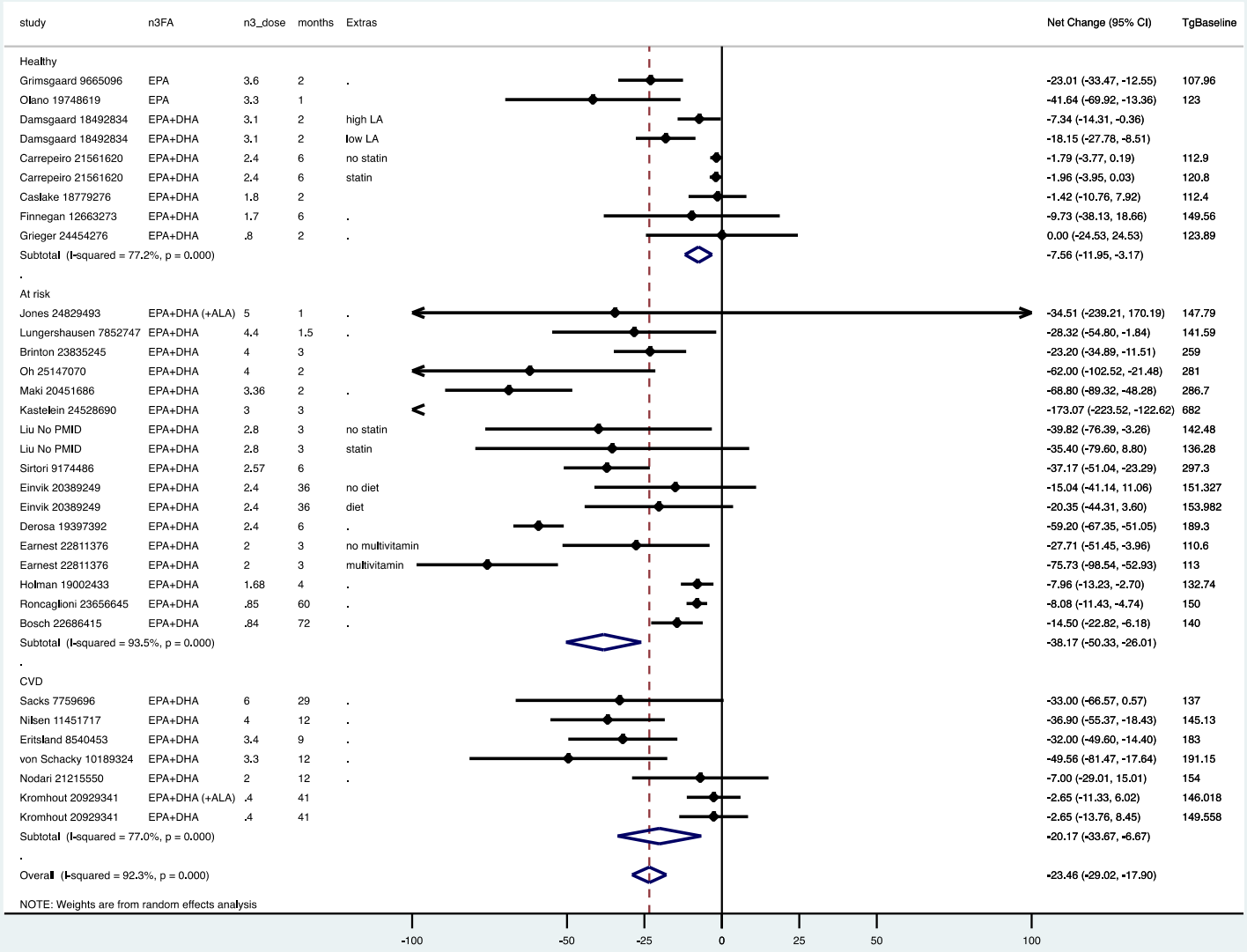
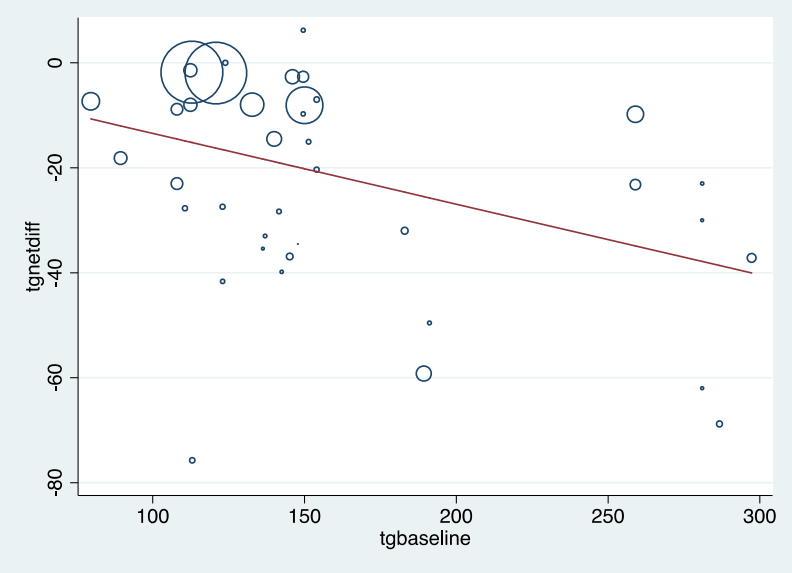
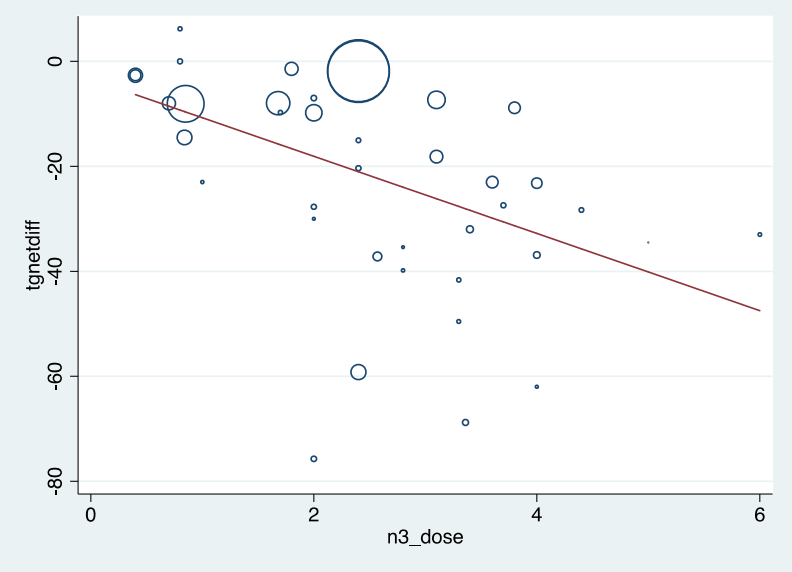


Figure AF.3. Metaregression of effect of EPA+DHA on net change Tg, by mean baseline Tg



Association of mean baseline Tg (tgbaseline) in mg/dL on net change Tg (tgnetdiff) in mg/dL. Circle sizes are related to each study's inverse variance.

Figure AF.4. Metaregression of effect of EPA+DHA on net change Tg, by EPA+DHA dose



Association of EPA+DHA dose (nd_dose) in g/d on net change Tg (tgnetdiff) in mg/dL. Circle sizes are related to each study's inverse variance.

Total cholesterol to HDL-c ratio

Randomized Controlled Trials

Eight RCTs provided data on effect of n-3 FA on the ratio of total cholesterol to HDL-c (Total:HDL-c) (**Table AG.1**),[78, 91, 110, 111, 114, 124, 125, 166] one in a healthy population, six in people at increased risk for CVD, and one in patients with CVD.

Total n-3 FA vs. placebo

Two trials compared total n-3 FA (ALA+EPA+DHA) versus placebo, following 2708 patients for 1 and 40 months; one in people at increased risk for CVD,[110] one in people with CVD.[114] Doses of ALA+EPA+DHA included ALA 1.2 g/d and EPA+DHA+DPA 5 g/d, and ALA 2 g/d and EPA+DHA 0.4 g/d. Baseline Total:HDL-c ratio was 4.0 in one trial and not reported in the other. Compliance was measured in both studies, but not reported. The estimates of the net differences were not significant, with wide confidence intervals.

Marine oil vs. placebo

Seven trials compared marine oil supplementation to placebo.[78, 91, 110, 111, 114, 124, 125] Five of seven trials found statistically significant reductions in Total:HDL-c ratios. Across populations, by meta-analysis, the summary net difference in Total:HDL-c ratio with EPA+DHA versus placebo was a statistically significant −0.26 (95% CI −0.41, −0.11) (**Figure AG.2**). Across studies, by metaregression, effect sizes did not statistically differ by population (at risk P=0.57, CVD P=0.61), marine oil dose (P=0.67), or baseline ratio (P=0.16).

Healthy population

One trial compared 2 months of both EPA 3.8 g/d and, separately, DHA 3.6 g/d to placebo in 224 healthy participants, total.[91] Compliance was assessed with pill count. The baseline Total:HDL-c ratio in the placebo group was 4.43. The trial found significant reductions with both marine oils compared to placebo (−0.2 and −0.3).

At risk for CVD population

Five trials compared EPA+DHA to placebo in 1185 people at increased risk for CVD.[78, 110, 111, 124, 125] Compliance was assessed by pill count or meal consumption in two trials. EPA+DHA dosages ranged from 1.5 to 5 g/d and followup ranged from 1 month to 3 years. Baseline Total:HDL-c ratios ranged from 4.29 to 4.7 in four trials and was 8.8 in one trial of patients (Kastelein 2014) with severe hypertriglyceridemia (≥500 mg/dL) at baseline.[111] All but one trial found a significant reduction in Total:HDL-c ratio. Net change Total:HDL-c ratio varied between −1.2 and −0.1. The pooled effect size was a statistically significant −0.38 (95% CI −0.52, −0.24). Exclusion of Katelein 2014 did not substantially affect the pooled estimate.

CVD population

One trial compared 3 months of 0.4 g/d EPA+DHA in patients with CVD.[114] Separate analyses were reported for patient taking statins or not. The study did not report compliance information. Baseline Total:HDL-c ratio data were also not reported. In both subgroups, no significant change in Total:HDL-c ratio was found, but there was a net increase in the ratio (0.09) in patients not taking statins and a net decrease in the ratio (−0.07) in those on statins.

RCT subgroup analyses

In the trial of patients with CVD, there was no apparent difference in effect on Total:HDL-c ratio based on cointervention with statins.[114] In a trial of people at increased risk of CVD, there was no interaction between EPA+DHA and general diet counseling.

Marine oil, comparison of different doses

As noted, the trial of people with severe hypertriglyceridemia compared three doses of EPA+DHA (3, 2.25, and 1.5 g/d).[111] At 3 month followup, the net differences among the three doses were not significantly different from each other. A second trial, comparing 2 and 4 g/d of total oil (of EPA+DHA) also found no significant differences in effect between the two doses at 1.5 months.[125] Across studies, no statistical difference in effect by dose was found by metaregression.

ALA vs. placebo

Two trials evaluated ALA versus placebo.[110, 114] In a trial of people at increased risk for CVD, no significant effects of ALA (both 1.4 and 5.9 g/d) were found on Total:HDL-c ratios at 1 month in 390 participants.[110] No difference in effect between the two doses was found in this trial. Similarly no significant effects were found in a trial of 2 g/d ALA in 2088 people with CVD at 3.4 years.[114]

Comparison of different specific n-3 FA

Grimsgaard 1998 compared EPA 3.8 g/d and DHA 3.6 g/d in 157 healthy people for 2 months. No difference in net change Total:HDL-c ratio was found.[91] Tatsuno 2013 compared two doses of EPA+DHA (3.36 and 1.68 g/d) to EPA 1.8 g/d alone.[166] Again, no differences in effect on Total:HDL-c ratio were found.

Observational Studies

Observational studies did not evaluate Total:HDL-c ratio.

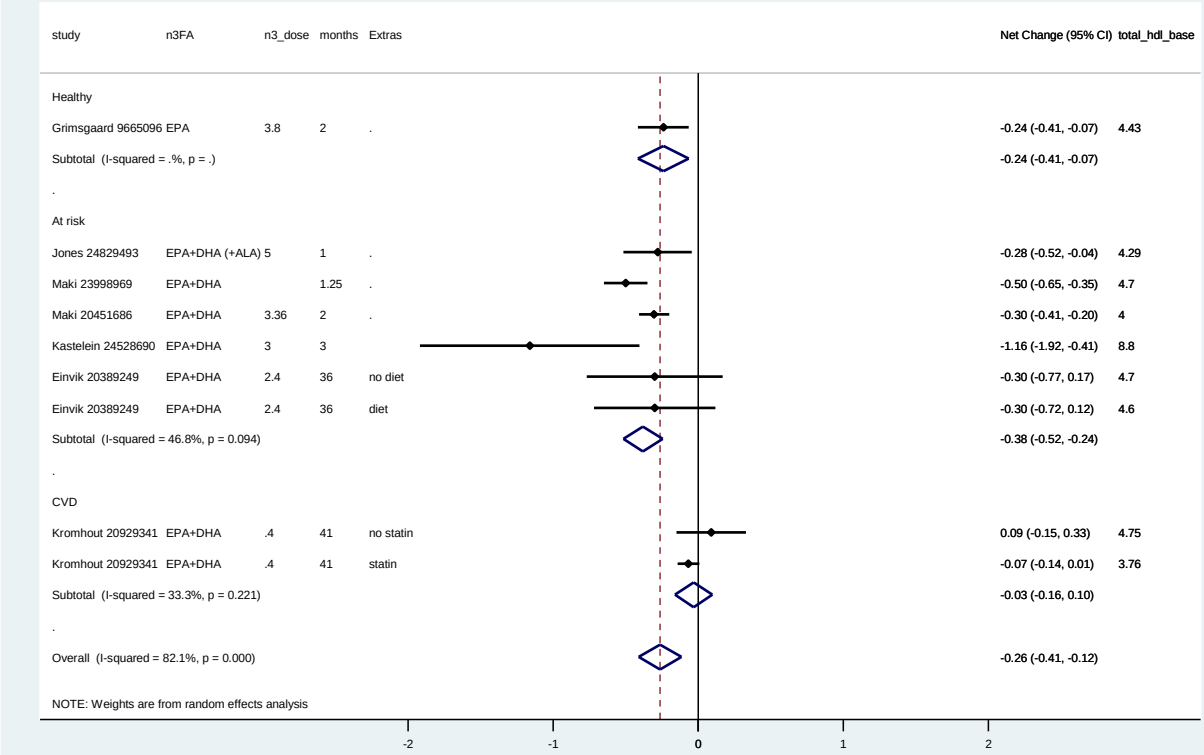
Table AG.1. Total cholesterol to HDL-c ratio: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL	Reported P value
Total n-3 FA vs Placebo													
Jones 2014 24829493 Canada	At risk	ALA+EPA+DHA	3.5 g/d (suppl)	Placebo	ALA 0.2 g/d (Canola oil)	1 mo	Meal consumption	130	4.01	130	4.24	-0.2 (-0.5, 0.05)	<0.05
Kromhout 2010 20929341 Scandinavia	CVD	ALA+EPA+DHA	2.4 g/d (Marine, Plant oil)	Placebo	0	3.4 y	nd	96	nd	113	nd	0.14 (-0.11, 0.39)	
		ALA+EPA+DHA + Statin	2.4 g/d (Marine, Plant oil)	Placebo + Statin	0	3.4 y	nd	947	nd	943	nd	-0.04 (-0.11, 0.04)	
Marine oil vs Placebo													
Grimsgaard 1998 9665096 Scandinavia	Healthy	EPA	3.8 g/d (Marine oil)	Placebo	0	2 mo	Pill count	75	4.70	77	4.43	-0.2 (-0.4, -0.1)	0.007
	Healthy	DHA	3.6 g/d (Marine oil)	Placebo	0	2 mo	Pill count	72	4.62	77	4.43	-0.3 (-0.5, -0.1)	0.0006
Einvik 2010 20389249 Scandinavia	At risk	EPA+DHA	2.4 g/d (Marine oil) [E:D 1.4]	Placebo	0	3 y	Pill count	70	4.8	68	4.7	-0.3 (-0.8, 0.2)	
		EPA+DHA + diet intervention	2.4 g/d (Marine oil) [E:D 1.4]	Placebo + diet intervention	0	3 y	Pill count	69	4.8	71	4.6	-0.3 (-0.7, 0.1)	
Kastelein 2014 24528690 World	At risk	EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	Placebo	0	3 mo	nd	99	9.0	98	8.8	-1.2 (-1.9, -0.4)	<0.01
		EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	Placebo	0	3 mo	nd	97	8.9	98	8.8	-0.7 (-1.5, 0)	<0.05
		EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	Placebo	0	3 mo	nd	99	8.8	98	8.8	-1.0 (-1.8, -0.3)	<0.05
Maki 2010 20451686 USA	At risk	EPA+DHA (+simvastatin)	3.36 g/d (Marine oil) [E:D 1.24]	Placebo (+simvastatin)	0	2 mo	nd	122	4.0	132	4.3	-0.3 (-0.4, -0.2)	<0.001

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL	Reported P value
Maki 2013 23998969 USA	At risk	EPA+DHA	4 g/d total oil (free fatty acid oil) [nd]	Placebo	0	1.5 mo	nd	207	4.9	211	4.7	-0.2 (-0.3, -0.1)	<0.001
		EPA+DHA	2 g/d total oil (free fatty acid oil) [nd]	Placebo	0	1.5 mo	nd	209	4.8	211	4.7	-0.1 (-0.2, 0.05)	NS
Jones 2014 24829493 Canada	At risk	EPA+DHA (+ALA)	EPA: 0.1 g/d, DHA: 3.5 g/d, DPA: 1.4 g/d (canola+DHA)	(ALA)	0	1 mo	Meal consumption	130	4.01	130	4.29	-0.3 (-0.5, -0.05)	<0.05
Kromhout 2010 20929341 Scandinavia	CVD	EPA+DHA	0.4 g/d (Marine oil) [E:D 1.5]	Placebo	0	3.4 y	nd	102	nd	113	nd	0.09 (-0.15, 0.33)	
		EPA+DHA + Statin	0.4 g/d (Marine oil) [E:D 1.5]	Placebo + Statin	0	3.4 y	nd	920	nd	943	nd	-0.07 (-0.14, 0.01)	
Marine oil vs marine oil (doses)													
Kastelein 2014 24528690 World	At risk	EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	3 mo	nd	99	9.0	97	8.9	-0.4 (-1.1, 0.3)	
		EPA+DHA	3 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	nd	99	9.0	99	8.8	-0.1 (-0.9, 0.6)	
		EPA+DHA	2.25 g/d (Marine oil) [E:D 2.75]	EPA+DHA	1.5 g/d (Marine oil) [E:D 2.75]	3 mo	nd	97	8.9	99	8.8	0.3 (-0.5, 1.0)	
Maki 2013 23998969 USA	At risk	ALA+EPA+ DHA	4 g/d total oil (free fatty acid oil) [nd]	ALA+EPA+ DHA	2 g/d total oil (free fatty acid oil) [nd]	1.5 mo	nd	207	4.9	209	4.8	-0.1 (-0.2, 0.05)	

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL	Reported P value
Marine oil vs Marine oil (miscellaneous)													
Grimsgaard 1998 9665096 Scandinavia	Healthy	EPA	3.8 g/d (Marine oil)	DHA	3.6 g/d (Marine oil)	2 mo	Pill count	72	4.62	75	4.70	0.1 (-0.1, 0.2)	0.4
Tatsuno 2013 24314359 Japan	At risk	EPA+DHA	3.36 g/d (Marine oil) [E:D 1.24]	EPA	1.8 g/d (Marine oil)	1 y	nd	170	nd	167	nd	-1.4 (-4.9, 2.1)	
		EPA+DHA	1.68 g/d (Marine oil) [E:D 1.24]	EPA	1.8 g/d (Marine oil)	1 y	nd	165	nd	167	nd	-0.9 (-3.9, 2.2)	
ALA vs Placebo													
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (Canola oil)	Placebo	ALA 0.2 g/d (Canola oil)	1 mo	Meal consumption	130	4.29	130	4.24	0.15 (-0.18, 0.48)	
		ALA	1.4 g/d (Canola Oleic oil)	Placebo	ALA 0.2 g/d (Canola oil)	1 mo	Meal consumption	130	4.29	130	4.24	0.16 (-0.17, 0.49)	
Kromhout 2010 20929341 Scandinavia	CVD	ALA	2 g/d (Plant oil)	Placebo	0	3.4 y	nd	102	nd	113	nd	0.07 (-0.17, 0.31)	
		ALA + Statin	2 g/d (Plant oil)	Placebo + Statin	0	3.4 y	nd	930	nd	943	nd	0.06 (-0.02, 0.13)	
ALA vs ALA (doses)													
Jones 2014 24829493 Canada	At risk	ALA	5.9 g/d (Canola oil)	ALA	1.4 g/d (Canola Oleic oil)	1 mo	Meal consumption	130	4.29	130	4.29	0 (-0.2, 0.2)	NS

Figure AG.2. Total cholesterol to HDL-c ratio: Randomized trials of marine oils



LDL-c to HDL-c ratio

Randomized Controlled Trials

Three RCTs provided data on effect of n-3 FA on the ratio of LDL-c to HDL-c (LDL:HDL-c) (**Table AH.1**),[121, 159, 166] all in people at increased risk for CVD.

Marine oil vs. placebo

Liu 2003 compared 2.8 g/d of EPA+DHA to placebo in 88 people at increased risk for CVD.[121] Baseline LDL:HDL-c ratio was about 3.1. Analyses were reported separately for a factorial analysis with simvastatin. At 3 month followup, no effect of LDL:HDL-c ratio was found with EPA+DHA supplementation in either subgroup, with no difference in effect regardless of simvastatin cotreatment.

Shidfar 2003 compared 0.81 g/d of EPA+DHA to placebo in 68 people at increased risk for CVD.[159] Baseline LDL:HDL-c ratio was about 4.2. Analyses were reported separately for a factorial analysis with vitamin C. At 2.5 month followup, no effect of LDL:HDL-c ratio was found with total n-3 FA supplementation in either subgroup, with no difference in effect regardless of vitamin C cosupplementation.

Tatsuno 2013 (in a trial without a placebo arm) compared 3.36 and 1.68 g/d EPA+DHA in 335 people at increased risk for CVD.[166] At 3 month followup, no significant difference in change in LDL:HDL-c ratio was found.

Comparison of different specific n-3 FA

Tatsuno 2013 compared 3.36 and 1.68 g/d EPA+DHA and 1.8 g/d EPA in 502 people at increased risk for CVD.[166] At 3 month followup, no significant differences in change in LDL:HDL-c ratios were found.

Observational Studies

Observational studies did not evaluate Total:HDL-c ratio.

Table AH.1. LDL-c to HDL-c ratio: RCTs

Study Year PMID Region	Population	Int (n-3 FA)	Int n-3 Dose (Source) [E:D; n-6:3]	Control	Ctrl n-3 Dose (Source) [E:D; n-6:3]	F/up Time	Compliance Verification	Int N	Int Baseline, mg/dL	Ctrl N	Ctrl Baseline, mg/dL	Net Chg, mg/dL	Reported P value
Marine oil vs Placebo													
Liu 2003 Scandinavia	At risk	EPA+DHA	2.8 g/d (Marine oil) [E:D 1.55]	Placebo	0	3 mo	Pill count	29	3.20	22	3.11	-0.02 (-0.45, 0.41)	NS
		EPA+DHA+ simvastatin	2.8 g/d (Marine oil) [E:D 1.55]	Placebo + simvastatin	0	3 mo	Pill count	19	3.28	18	3.02	-0.1 (-0.7, 0.5)	NS
Shidfar 2003 12847992 Iran	At risk	EPA+DHA	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo	0	2.5 mo	nd	16	4.42	19	4.2	-0.3 (-1.5, 0.9)	
		EPA+DHA+ vitamin C	EPA 0.5 g/d, DHA 0.31 g/d (suppl) [E:D 1.6]	Placebo + vitamin C	0	2.5 mo	nd	16	4.4	17	4.3	0.2 (-1.1, 1.5)	
Marine oil vs Marine oil (doses)													
Tatsuno 2013 24314359 Japan	At Risk	EPA+DHA	EPA: 1.86 g/d, DHA: 1.50 g/d (Marine oil)	EPA+DHA	EPA: 0.93 g/d, DHA: 0.75 g/d (Marine oil)	12 wk	Pill count	170	nd	165	nd	2.6% (-1.5, 6.7)	
Marine oil vs Marine oil (miscellaneous)													
Tatsuno 2013 24314359 Japan	At risk	EPA+DHA	3.36 g/d (Marine oil) [E:D 1.24]	EPA	1.8 g/d (Marine oil)	1 y	nd	170	nd	167	nd	1.8% (-2.4, 5.9)	
		EPA+DHA	1.68 g/d (Marine oil) [E:D 1.24]	EPA	1.8 g/d (Marine oil)	1 y	nd	165	nd	167	nd	-0.9% (-4.5, 2.8)	

Summary by n-3 FA

The trials of clinical outcomes were almost all conducted in populations at increased risk of CVD, largely related to dyslipidemia, or with CVD. The trials that reported intermediate outcomes (BP and lipoproteins), were conducted in generally healthy, at-risk, and CVD populations. The observational studies, in contrast, were almost all conducted in general (unrestricted by CVD or risk factors) or healthy populations. Observational studies did not analyze intermediate CVD outcomes.

Total n-3 FA (ALA+EPA+DHA)

Overall, there is insufficient evidence regarding the effect of or association between total n-3 FA and clinical or intermediate outcomes (**Table EP.1**). There is low strength of evidence of no association between total n-3 FA intake and stroke death, and total (fatal and nonfatal) MI (each association based on longitudinal observational studies).

Clinical event outcomes, RCTs

No RCTs reported clinical event outcomes for comparisons of total n-3 FA versus placebo.

Intermediate outcomes, RCTs

Two RCTs that evaluated BP compared combined ALA and marine oil (ALA 1.2 g/d [canola oil] or 2 g ["plant oil"], and 3.6 or 0.4 g EPA+DHA) versus placebo reported on intermediate outcomes. Neither trial found significant effects on BP, LDL-c, HDL-c, Tg, or Total:HDL-c ratio.

Observational studies, intake

Seven studies evaluated total n-3 FA intake. For each outcome there was no consistent (and replicated) significant association between total n-3 FA intake and risk reduction. One of three studies found a significant association between higher total n-3 FA intake and *higher* risk of MACE. In contrast, one of three studies found an association with reduced risk of CVD death; one of two studies found a significant association with MI death; one study each found significant associations with lower risk of ischemic stroke death and CHF death. No studies found significant associations with all-cause death (1 study), CHD death (2 studies), total (ischemic and hemorrhagic) stroke death (3 studies), MI (1 study), total (fatal and nonfatal) stroke (1 study), SCD (1 study), or incident HTN (1 study).

One study found no significant difference in association of total n-3 FA with total CVD death between men and women or by amount of fish consumption. Another study found no significant difference in association with MI death, total stroke death, or ischemic stroke death by baseline Total:HDL-c ratio.

Observational studies, biomarkers

Three studies evaluated biomarkers for total n-3 FA (combined; plasma, blood, or erythrocyte). One study evaluated numerous outcomes and found significant associations between higher biomarker level and reduced risk of most outcomes (CVD death, CHD death, all-cause death, CHD, ischemic stroke, SCD, AFib, and CHF), but not stroke death, total stroke, or

hemorrhagic stroke. In contrast, a second study found no significant association with CHD. The third study found no significant association overall with incident HTN, but did find a significant association in between higher total n-3 FA and HTN in younger women (<55 years old) but not in older women.

Table EP.1. Evidence profile for the effect and association of total n-3 FA with CVD outcomes*

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Major adverse cardiovascular events (MACE)	Insufficient	RCT: 0 Obs intake: 3 Obs biomarkers: 0	Low	RCT: NA Obs intake: Inconsistent Obs biomarkers: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: NA	No RCT	RCT: NA Obs intake: Unclear Obs biomarkers: NA
CVD death (including stroke)	Insufficient	RCT: 0 Obs intake: 3 Obs biomarkers: 1	Low	RCT: NA Obs intake: Inconsistent Obs biomarkers: NA All: Inconsistent	RCT: NA Obs intake: Precise Obs biomarker: Precise	No RCT	RCT: NA Obs intake: Unclear Obs biomarkers: Lower risk
Cardiac death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 0	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarkers: NA
Coronary heart disease death	Insufficient	RCT: 0 Obs intake: 2 Obs biomarkers: 1	Moderate	RCT: NA Obs intake: Consistent Obs biomarkers: NA All: inconsistent	RCT: NA Obs intake: Imprecise Obs biomarker: Precise	Sparse	RCT: NA Obs intake: No association Obs biomarkers: Lower risk
Myocardial infarction death	Insufficient	RCT: 0 Obs intake: 2 Obs biomarkers: 0	Moderate	RCT: Obs intake: Inconsistent Obs biomarkers All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: Unclear Obs biomarkers: NA
Heart failure death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 0	Moderate	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Unclear	Sparse	RCT: NA Obs intake: Lower risk Obs biomarkers: NA
Stroke death	Low	RCT: 0 Obs intake: 3 Obs biomarkers: 1	Low	RCT: NA Obs intake: Consistent Obs biomarkers: NA All: Consistent	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	No RCT	RCT: NA Obs intake: No association Obs biomarkers: No association
Ischemic stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 0	Moderate	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Unclear Obs biomarker: NA	Sparse	RCT: NA Obs intake: Lower risk Obs biomarkers: NA
Hemorrhagic stroke death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 0	NA	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarkers: NA
Death, all-cause	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 1	Moderate	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Precise Obs biomarker: Precise	Sparse	RCT: NA Obs intake: No association Obs biomarkers: Lower risk

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Coronary heart disease	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 2	Low	RCT: NA Obs: NA Obs biomarkers: Inconsistent All: Consistent	RCT: NA Obs intake: NA Obs biomarker: Precise	Sparse	RCT: NA Obs intake: NA Obs biomarkers: Unclear
Myocardial infarction	Low	RCT: 0 Obs intake: 3 Obs biomarkers: 0	Low	RCT: NA Obs intake: Consistent Obs biomarkers: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: NA	No RCT	RCT: NA Obs intake: No association Obs biomarkers: NA
Acute coronary syndrome	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 0	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Unclear Obs biomarkers: NA	Sparse	RCT: NA Obs intake: No association Obs biomarkers: NA
Angina pectoris	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 0	NA	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarkers: NA
Atrial fibrillation	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: NA Obs biomarkers: Unclear	Sparse	RCT: NA Obs intake: NA Obs biomarkers: Lower risk
Congestive heart failure	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 1	Low	RCT: NA Obs: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: NA Obs biomarkers: Unclear	Sparse	RCT: NA Obs intake: NA Obs biomarkers: No association
Stroke, total	Insufficient	RCT: 0 Obs: 1 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: Consistent	RCT: NA Obs intake: Imprecise Obs biomarkers: Imprecise	Sparse	RCT: NA Obs intake: No association Obs biomarkers: No association
Stroke, ischemic	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: NA Obs biomarkers: Imprecise	Sparse	RCT: NA Obs intake: NA Obs biomarkers: Lower risk
Stroke, hemorrhagic	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: NA Obs biomarkers: Imprecise	Sparse	RCT: NA Obs intake: NA Obs biomarkers: No association
Sudden cardiac death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: Consistent	RCT: NA Obs intake: Imprecise Obs biomarkers: Imprecise	Sparse	RCT: NA Obs intake: No association Obs biomarkers: No association

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Revascularization	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 0	NA	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarkers: NA
Hypertension	Insufficient	RCT: 0 Obs intake: 2 Obs biomarkers: 0	Low	RCT: NA Obs intake: Inconsistent Obs biomarkers: NA All: NA	RCT: NA Obs intake: Precise Obs biomarker: NA	Sparse	RCT: NA Obs intake: Unclear Obs biomarkers: NA
Blood pressure (SBP, DBP, MAP combined)	Insufficient	RCT: 1 Obs intake: 0 Obs biomarkers: 0	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarkers: NA
LDL-c	Insufficient	RCT: 2 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	No Obs	RCT: No effect Obs intake: NA Obs biomarkers: NA
HDL-c	Insufficient	RCT: 2 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	No Obs	RCT: No effect Obs intake: NA Obs biomarkers: NA
Triglycerides	Insufficient	RCT: 2 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Inconsistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	No Obs	RCT: Unclear Obs intake: NA Obs biomarkers: NA
HDL-c/Total cholesterol to LDL-c ratios	Insufficient	RCT: 2 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	No Obs	RCT: No effect Obs intake: NA Obs biomarkers: NA

* No reporting bias was detected for any outcome. All studies that measured n-3 FA intake were assessed to be direct, while all biomarker studies were assessed to be indirect.
Abbreviations: DBP = diastolic blood pressure, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MAP = mean arterial pressure, NA = not applicable, Obs = observational study, RCT = randomized controlled trial, SBP = systolic blood pressure.

Marine oil, total: EPA+DHA±DPA

Overall, there is moderate to high strength of evidence of beneficial effects of increased marine oil intake for selected CVD and intermediate outcomes, but low to high strength of evidence for no effect or association of higher intake and other selected CVD and intermediate outcomes (**Table EP.2**). There is insufficient evidence for most outcomes of interest. More specifically, there is high strength of evidence of that marine oils clinically and statistically significantly lower Tg—possibly with greater effects with higher doses and in people with higher baseline Tg—and statistically, but arguably not clinically, significantly raises HDL-c. There is also high strength of evidence that marine oil significantly lowers Total:HDL-c ratio. There is moderate strength of evidence that marine oil supplementation lowers risk of MACE and total CVD death. There is a high strength of evidence of no effect of marine oil on risk of total stroke, but low strength of evidence of no associations of marine oil intake and ischemic or hemorrhagic stroke. There is low strength of evidence for associations between higher EPA+DHA intake and decreased risk of CHD (up to an intake dose of about 1 g/d) and CHF (up to an intake dose of only 0.2 g/d), based on observational studies. However, there is moderate to high strength of evidence of no effect of (or association between) marine oil and all-cause death, MI, AFib, CHF, sudden cardiac death, revascularization, BP, LDL-c, or LDL:HDL-c ratio. There is also low strength of evidence of no effect of marine oil intake and CHD death. There is insufficient evidence for other outcomes.

Clinical event outcomes, RCTs

Regarding clinical event outcomes, 18 trials in populations at increased risk for CVD (2 RCTs) and CVD populations (16 RCTs) mostly found no significant effects of marine oil (EPA+DHA±DPA) versus placebo on specific clinical event outcomes. Across RCTs, EPA+DHA doses ranged from 0.34 to 6 g/d (median 0.866 g/d). Followup ranged from 1 to over 10 years (median 3.9 years).

Two of 15 trials found significantly lower risk of all-cause death with EPA+DHA (both 0.866 g/d; HR = 0.79 and 0.91), however, the meta-analyzed HR was nonsignificant at 0.97 (95% CI 0.90, 1.05) with no differences across trials by marine oil dose, followup time, or population (CVD, at risk, healthy). Four trials also found no within-study subgroup differences in effect on death for multiple subgroup comparisons.

Eight RCTs each reported on both MACE and total MI, only one of which found a significant reduction in outcome with 0.866 g/d EPA+DHA at 3.9 year followup (HR=0.92, both outcomes). Meta-analysis of MACE (which included a ninth trial of EPA) found a just-significant association (HR=0.95; 95% CI 0.90, 1.00; P=0.047) with no significant differences across studies by marine oil dose (range 0.4-2 g/d), followup time (range 1-5 y), or population category. Within-study subgroup analyses found a significant effect in women but not men in one trial, but no significant difference in effect between sexes in a second trial, and no differences between multiple subgroups in three trials. Meta-analysis of MI (also with the EPA trial) was nonsignificant (HR=0.93; 95% CI 0.83, 1.04), with no significant differences across studies by marine oil dose, followup time, or population category. In one trial, no significant difference in effect was found based on cointervention with B vitamins.

Two of six RCTs found significant effects of 0.866 g/d marine oil (EPA+DHA) on risk of CVD death in populations of people with existing CVD. By meta-analysis, there was a near-

significant effect (HR=0.91; 95% CI 0.81, 1.01; P=0.073), with no significant differences across studies by marine oil dose, followup time, or population.

Eight RCTs all found no significant effect of EPA+DHA with SCD; by meta-analysis (with the EPA trial), summary HR=1.02 (95% CI 0.92, 1.14). Six RCTs also found no significant effect of marine oils with total stroke; by meta-analysis, summary HR=1.02 (95% CI 0.88, 1.19).

All EPA+DHA RCTs that evaluated revascularization (5 trials), CHD death (4 trials), total stroke death (3 trials), AFib (3 trials, and CHF death (1 trial) found no significant effect of marine oils. One trial found an effect in participants with diabetes that was not seen in those without diabetes, but no test of interaction was reported. Two trials compared effect of marine oils on AFib in multiple subgroups, finding no significant differences.

Four EPA+DHA RCTs found inconsistent effects on cardiac death, with effect sizes ranging from 0.45 to 1.45. One trial found a statistically significant *reduction* in cardiac death with 0.866 g/d EPA+DHA at 3.5 years (RR=0.65; 95% CI 0.51, 0.82); one trial found a statistically significant *increase* in cardiac death with a fish diet with EPA+DHA supplements (0.855 g/d EPA+DHA; HR=1.45; 95% CI 1.05, 1.99), but no significant effect on cardiac death among people only given advice to increase fish intake (by 0.45 g/d EPA+DHA) or in two other trials of 0.96 and 2.6 g/d EPA+DHA. The trial that found increased risk with combined fish diet and EPA+DHA supplementation found no significant difference in effect between multiple sets of subgroups based on drug cointervention.

One of three EPA+DHA RCTs each found significant effects of reduced angina and CHF incidence. For angina, across studies EPA+DHA doses ranged from 1.8 to 6 g/d and effect sizes ranged from 0.64 to 1.18; the one trial with a significant effect used a dose of 1.8 g/d. For CHF, across studies doses ranged from 0.866 to 6 g/d and effect sizes ranged from 0.67 to 0.86 (one trial had only one participant who developed CHF); the one trial that found a significant reduction in CHF incidence used a dose of ≥0.85 g/d.

Intermediate outcomes, RCTs

Twenty-two RCTs that compared EPA+DHA to placebo evaluated systolic BP, of which 20 also reported on diastolic BP. Six RCTs were in healthy populations, 11 in those at risk for CVD, and five in those with CVD. All trials found no significant difference in BP across EPA+DHA doses of 0.30 to 6 g/d and followup durations of 1 month to 6 years. By meta-analysis (together with two trials of EPA or DHA alone), no significant effects on systolic (summary net difference = 0.15 mmHg; 95% CI −0.17, 0.47) or diastolic (summary net difference = −0.06 mmHg; 95% CI −0.32, 0.21) BP were found. Three of the trials also found no effect on MAP. By meta-regression, no differences in effect across studies were found by marine oil dose, followup duration or population. Three trials directly compared different EPA+DHA doses and found no differences in effect (1.7 vs. 0.8 g/d; 1.8 vs. 0.9 or 0.45 g/d; 3.4 vs. 1.7 g/d). One trial found no difference in effect between people with normal BP or prehypertension.

Numerous included RCTs compared the effect of marine oils and placebo (or equivalent) on blood lipids. Thirty-three RCTs evaluated LDL-c and HDL-c. Marine oil doses ranged from 0.3 to 6 g/d (median 2.4 g/d) and study followup times ranged from 1 month to 6 years (median 3 months). Meta-analysis of the effect of marine oils on LDL-c found no significant effect (summary net change = 0.3 mg/dL; 95% CI −0.7, 1.2). In contrast, marine oils increased HDL-c by a small, statistically significant amount (summary net change = 1.2 mg/dL; 95% CI 0.6, 1.8). For both lipoprotein fractions, no significant differences in effect across studies were found by marine oil dose, followup duration or population. Seven studies found no significant differences

in effect within study by EPA+DHA dose. For HDL-c, three trials found no significant difference in effect between people using statins or not; one or two trials, each, found no significant differences between subgroups based on sex or age. One trial found a larger HDL-c effect in a subgroup also randomized to an exercise regimen; one of two trials found a larger HDL-c effect in people with impaired glucose tolerance compared to those with normoglycemia. Seven trials found mostly nonsignificant effects of marine oil (0.4-5 g/d for 1 month to 3 years) on Total:HDL-c ratio; the one trial in healthy participants found significant reductions (−0.5 and −0.8, depending on specific marine oil). The single trial of people with severe hypertriglyceridemia (baseline >500 mg/dL), with subsequent atypically high Total:HDL ratio (8.8), found significant reductions in the ratio with EPA+DHA supplementation (−0.8 and −1.8, depending on dose). The other five trials found no significant net changes in Total:HDL ratio (−0.2 or −0.3 in three at risk populations; −0.06 in people with CVD). The trial of purified EPA and purified DHA supplementation found no difference in effect between the two n-3 FA; the trial comparing different EPA+DHA doses also found no differences in effect among them. One trial of 2.8 g/d EPA+DHA found no significant effect on LDL:HDL-c ratio; another trial found no significant difference in change in ratio between 3.4 and 1.7 g/d EPA+DHA.

Thirty-four included RCTs mostly found significant effects of marine oils (0.3-6 g/d; median 2.4 g/d for 1 month to 6 years; median 3 months) on Tg levels. Meta-analysis found a summary net change of −23 mg/dL (95% CI −29, −18), with no significant difference in effect based on population or followup time across studies. By metaregression, each increase in mean baseline Tg concentration by 1 mg/dL was associated with a greater net decrease in Tg concentration of −0.12 mg/dL (95% CI −0.22, −0.03; P=0.013); each increase of EPA+DHA dose by 1 g/d was also associated with a greater net decrease in Tg concentration of −6.8 mg/dL (95% CI −11.4, −2.2; P=0.005). No clear inflection point was found at any dose. Five of six trials found no significant difference in Tg change by EPA+DHA dose, but across trials all doses of 3.4 and 4 g/d lowered Tg concentration by at least 30 mg/dL more than lower doses (1-2 g/d), while all pairwise comparisons of lower doses (1.7-3 g/d) to even lower doses (0.7-2.25 g/d) found much smaller differences between doses (−17 to 6 mg/dL). Two trials both found significantly larger Tg concentration lowering effects of EPA (3.6 or 3.3 g/d) than DHA (3.8 or 3.7 g/d). No significant differences were found based on statin use (4 trials), vitamin C use (1 trial), concurrent high or low linoleic acid diet (1 trial), concurrent general dietary advice (1 trial), or age (1 trial). One trial found a significantly larger effect on Tg among people also taking a multivitamin. One trial found a larger effect of higher dose EPA+DHA (1.8 g/d) in men than women, but no significant difference between sexes at 0.8 g/d. One trial found no significant difference in effect between people with impaired glucose tolerance and those with noninsulin dependent diabetes, but among those with diabetes, a larger effect was found in those with baseline HDL-c ≤35 mg/dL compared to higher levels.

Observational studies, intake

Twenty-one observational studies evaluated associations between total EPA+DHA±DPA intake (regardless of source) and numerous clinical outcomes. Only eight (38%) of these found significant associations with any clinical outcome. By meta-analysis, overall there is a near significant association between marine oil intake and CHD across a median dose range of 0.038 to 3.47 g/d; the best-fit curve found a change in slope (between g/d and risk of CHD) at 1.0 g/d. Below this threshold, increasing dose of marine oil was protective against CHD; above there is no significant association. However, using metaregression thresholds from 0.2 to 1.4 g/d resulted

in similar findings (protective associations at lower intake, no significant association at higher intake). By meta-analysis, there was no significant association for total stroke across a median dosage range of 0.025 to 0.6 g/d. By meta-analysis, there is a just-significant association between higher marine oil intake and *higher* risk of ischemic stroke across a median dosage range of 0.025 to 0.6 g/d, but with a best-fit threshold at 0.3 g/d with a nonsignificant decreasing risk of with higher intake below this threshold and a nonsignificant increasing risk above it. Similar results were found with thresholds between 0.1 and 0.5 g/d. By meta-analysis, no significant association was found between EPA+DHA±DPA intake and hemorrhagic stroke. No studies found significant associations between intake and all-cause death (2 studies).

A minority of studies found significant associations of decreased risk of other outcomes with increasing intake of EPA+DHA±DPA: MACE (1/2 studies), all-cause death (1/3 studies), CVD death (1/4 studies), CHD death (3/7 studies), MI (1/2 studies), incident CHF (1/5 studies), and AFib (1/3 studies). No studies found significant associations with cardiac death (1 study), total stroke death (1 study), ischemic stroke death (1 study), coronary revascularization (1 study), SCD (2 studies), and incident HTN (1 study). One study each analyzed MI death and ischemic stroke death and found a significant association.

Observational studies, biomarkers

Five studies evaluated combined EPA+DHA±DPA biomarkers, including adipose tissue, cholesteryl ester, erythrocyte, phospholipid, and plasma n-3 FA levels. Of the outcomes evaluated, none was analyzed by more than two studies. One study each found no significant association between various biomarker levels and MI, hemorrhagic stroke, total stroke (with a P value of 0.07), or cardiac death. One study found a significant association between higher phospholipid EPA+DHA+DPA and incident CHD. Another found a significant association between higher adipose EPA+DHA+DPA and ACS in men, but not in women. Two studies each evaluated CHF, ischemic stroke, and MACE. For each outcome only one of the studies found significant associations with EPA+DHA±DPA biomarker levels. In one of the studies of CHF, phospholipid EPA+DHA+DPA level was associated with the outcome in women only but cholesteryl ester EPA+DHA+DPA levels were not associated in either sex.

Table EP.2. Evidence profile for the effect and association of marine oil (EPA+DHA± DPA) with CVD outcomes*

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Major adverse cardiovascular events (MACE)	Moderate	RCT: 8 Obs intake: 3 Obs biomarkers: 2	Low	RCT: Consistent Obs intake: Consistent Obs biomarkers: Inconsistent All: Inconsistent	RCT: Precise Obs intake: Precise Obs biomarker: Precise	None	RCT: Lower risk 0.95 (0.90, 1.00) Obs intake: No association Obs biomarkers: Unclear
CVD death (including stroke)	Moderate	RCT: 6 Obs intake: 4 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: Inconsistent Obs biomarkers: NA All: Inconsistent	RCT: Precise (NS) Obs intake: Imprecise Obs biomarker: NA	None	RCT: Lower risk 0.91 (0.81, 1.01) Obs intake: Unclear Obs biomarkers: NA
Cardiac death	Insufficient	RCT: 4 Obs intake: 1 Obs biomarkers: 1	Low	RCT: Inconsistent Obs intake: NA Obs biomarkers: NA All: Inconsistent	RCT: Precise Obs intake: Imprecise Obs biomarker: Imprecise	Sparse Obs	RCT: Unclear Obs intake: No association Obs biomarkers: No association
Coronary heart disease death	Low	RCT: 3 Obs intake: 7 Obs biomarkers: 0	Moderate	RCT: Consistent Obs intake: Inconsistent Obs biomarker: NA All: Inconsistent	RCT: Imprecise Obs intake: Imprecise Obs biomarker: NA	None	RCT: No effect Obs intake: Unclear Obs biomarkers: NA
Myocardial infarction death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 0	Low	RCT: NA Obs: NA Obs intake: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: NA	Sparse	RCT: NA Obs intake: Lower risk Obs biomarkers: NA
Heart failure death	Insufficient	RCT: 1 Obs intake: 0 Obs biomarkers: 0	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarkers: NA
Stroke death	Insufficient	RCT: 2 Obs intake: 1 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: Consistent	RCT: Imprecise Obs intake: Imprecise Obs biomarker: NA	Sparse	RCT: No effect Obs intake: No association Obs biomarkers: NA
Ischemic stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 0	Moderate	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Unclear Obs biomarker: NA	Sparse	RCT: NA Obs intake: Lower risk Obs biomarkers: NA
Hemorrhagic stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 0	Moderate	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Unclear Obs biomarker: NA	Sparse	RCT: NA Obs intake: No association Obs biomarkers: NA
Death, all-cause	High	RCT: 15 Obs intake: 3 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: Consistent Obs biomarkers: NA All: Inconsistent	RCT: Precise Obs intake: Precise Obs biomarker: NA	None	RCT: No effect 0.97 (0.91, 1.04) Obs: No association Obs biomarkers: NA

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Coronary heart disease	Low	RCT: 0 Obs intake: 7 Obs biomarkers: 1	Low	RCT: NA Obs intake: Consistent Obs biomarkers: NA All: Consistent	RCT: NA Obs intake: Precise Obs biomarker: Precise	No RCT	RCT: NA Obs intake: Lower risk <1 g/d, HR per g/d: 0.77 (0.65, 0.91) Obs biomarkers: Lower risk
Myocardial infarction	Moderate	RCT: 7 Obs intake: 1 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: Consistent	RCT: Precise (NS) Obs intake: Precise Obs biomarker: NA	Sparse Obs	RCT: No effect 0.89 (0.77, 1.03) Obs intake: No association Obs biomarkers: NA
Acute coronary syndrome	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: No association Obs biomarkers: No association
Angina pectoris	Insufficient	RCT: 2 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarkers: NA
Atrial fibrillation	Moderate	RCT: 3 Obs intake: 3 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: Inconsistent Obs biomarkers: NA All: Consistent	RCT: Precise Obs intake: Imprecise Obs biomarker: NA	Few studies	RCT: No effect Obs: Unclear Obs biomarkers: NA
Congestive heart failure	Low	RCT: 3 Obs intake: 5 Obs biomarkers: 2	Low	RCT: Consistent Obs intake: Consistent Obs biomarkers: Consistent All: Inconsistent	RCT: Precise Obs intake: Precise Obs biomarker: Imprecise	Few RCTs	RCT: No effect Obs intake: Lower risk <0.2 g/d, HR per g/d: 0.45 (0.28, 0.72) Obs biomarkers: No association
Stroke, total	High	RCT: 6 Obs intake: 4 Obs biomarkers: 2	Low	RCT: Consistent Obs intake: Consistent Obs biomarkers: Inconsistent All: Consistent	RCT: Precise Obs intake: Imprecise Obs biomarker: Imprecise	None	RCT: No effect 1.02 (0.88, 1.19) Obs intake: No association Obs biomarkers: Unclear
Stroke, ischemic	Low	RCT: 0 Obs intake: 4 Obs biomarkers: 2	Low	RCT: NA Obs intake: Consistent Obs biomarkers: Inconsistent All: Inconsistent	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	None	RCT: NA Obs intake: No association <0.3 g/d, HR per g/d: 0.77 (0.27, 2.16) Obs biomarkers: Unclear
Stroke, hemorrhagic	Low	RCT: 0 Obs intake: 4 Obs biomarkers: 1	Low	RCT: NA Obs intake: Consistent Obs biomarkers: NA All: Consistent	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	None	RCT: NA Obs intake: No association <0.3 g/d, HR per g/d: 0.62 (0.35, 1.10) Obs biomarkers: No association

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Sudden cardiac death	High	RCT: 8 Obs intake: 1 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: Consistent	RCT: Precise Obs intake: Imprecise Obs biomarkers: NA	None	RCT: No effect 1.02 (0.92, 1.14) Obs intake: No association Obs biomarkers: NA
Revascularization	High	RCT: 5 Obs intake: 1 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: Obs biomarker:	Sparse Obs	RCT: No effect Obs intake: No association Obs biomarkers: NA
Hypertension	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 0	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: Precise Obs biomarker: NA	Sparse	RCT: NA Obs intake: No association Obs biomarkers: NA
Blood pressure (SBP, DBP, MAP combined)	High	RCT: 22 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	No Obs	RCT: No effect SBP: 0.3 mmHg (-0.3, 0.8) DBP: -0.2 mmHg (-0.5, 0.1) Obs intake: NA Obs biomarkers: NA
LDL-c	High	RCT: 33 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	No Obs	RCT: No effect 0.25 mg/dL (-0.67, 1.17) Obs intake: NA Obs biomarkers: NA
HDL-c	High	RCT: 33 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	No Obs	RCT: Lower risk (raise HDL-c) 1.21 mg/dL (0.58, 1.84) Obs intake: NA Obs biomarkers: NA
Triglycerides	High	RCT: 34 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	No Obs	RCT: Lower risk (lower triglycerides) -23 mg/dL (-29, -18) Obs intake: NA Obs biomarkers: NA
HDL-c/Total cholesterol to LDL-c ratios	High	RCT: 7 Obs intake: 0 Obs biomarkers: 0	Low	RCT: Consistent Obs intake: NA Obs biomarkers: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	No Obs	RCT: Lower risk −0.26 (−0.41, −0.11) Obs intake: NA Obs biomarkers: NA

* No reporting bias was detected for any outcome. All studies that measured n-3 FA intake were assessed to be direct, while all biomarker studies were assessed to be indirect.
Abbreviations: DBP = diastolic blood pressure, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MAP = mean arterial pressure, NA = not applicable, Obs = observational study, RCT = randomized controlled trial, SBP = systolic blood pressure.

EPA

For the most part, there is insufficient evidence regarding the effect of, or association with, EPA (specifically) and CVD clinical and intermediate outcomes (**Table EP.3**). There is low strength of evidence of no association between EPA intake and CHD and between EPA biomarkers and AFib.

Clinical event outcomes, RCTs

Regarding clinical event outcomes, one trial in an at risk population (dyslipidemia), found that after 5 years, compared with placebo, people taking purified EPA 1.8 g/d had significantly lower risk of MACE and angina, but no significant difference in CHD death, coronary revascularization, SCD, or MI (also in the subgroup of people with prior CVD). Subgroup analysis for CHD death found no clear difference between those who also had CVD versus those without CVD.

Intermediate outcomes, RCTs

One trial of purified EPA 3.8 g/d versus placebo found no significant effect on systolic BP, diastolic BP, or MAP. This trial and another of EPA 3.3 g/d found no significant effect of EPA on LDL-c or HDL-c. Both trials, however, found significant net reductions in Tg concentration (−42 and −23 mg/dL). The trial of EPA 3.8 g/d also found a significant reduction in Total:HDL-c ratio (−0.2).

Observational studies, intake

Eight studies evaluated associations between estimated total EPA intake (specifically) and clinical outcomes. No outcome was evaluated by more than two studies. One study each found no significant association between EPA intake and ACS, ischemic stroke, or total stroke death. One study found a significant association between higher EPA intake and lower ischemic stroke death in healthy adults (in quantiles with median EPA intake >0.07 g/d in men and >0.06 g/d in women), but no association with hemorrhagic stroke death. One study found a significant association between higher EPA intake and lower risk of all-cause death (>0.01 g/d) in healthy adults. Another study found a significant association with MACE in healthy adults (>0.09 g/d). Two studies, each, found no significant associations between EPA intake and incident CHD (although P=0.06 in one) or CHD death. For both incident HTN and CVD death, one of two studies found significant associations between higher EPA (0.02 g/d for HTN and 0.01 g/d for CVD death) intake and lower risk of outcomes; the other studies found no such associations.

Observational studies, biomarkers

Ten studies evaluated associations between various EPA biomarkers and clinical outcomes. For three clinical outcomes, two of three studies found significant associations between higher EPA biomarker level and reduced risk of outcome. Three studies of healthy adults evaluated CHD, two of which found increased plasma or phospholipid EPA levels were associated with reduced CHD risk; the third study evaluated blood EPA levels. Three studies, two in healthy adults, one in people with hypercholesterolemia, evaluated MACE; the study of people with hypercholesterolemia found an association of reduced MACE risk with higher plasma EPA, as did one study of phospholipid EPA in healthy adults. The third study found no significant association between erythrocyte EPA and MACE in healthy adults. Three studies, two

in healthy adults, one in adults with a history of MI, evaluated CHF; in one study of healthy adults higher plasma EPA was associated with reduced CHF risk, but the other study of healthy adults found no association with phospholipid or cholesteryl ester EPA. The study in people with a history of MI also found an association with higher blood EPA. In this latter study, significant interactions were found for sex (no association was seen in women, in contrast with a significant association in men), statin use (those on statins had no association, in contrast with those on statins), and baseline HDL-c level (those with higher HDL-c had no association, in contrast with those with HDL-c <40 mg/dL). No interactions were found for age, use of angiotensin receptor blocker drugs, use of beta blocker drugs, diabetes, dyslipidemia, baseline LDL-c, hypertension, glomerular filtration function, or hypertriglyceridemia.

One of three studies found a significant association between higher EPA biomarkers (plasma EPA) and lower risk of death in healthy adults, but a second study of plasma EPA in healthy adults found no such association; nor did a study of blood EPA in people with a history of MI. One of two studies of plasma EPA in healthy adults found a significant association with CVD death. Two studies found no significant association between EPA biomarkers and ischemic stroke. One study found a significant association between erythrocyte EPA and incident HTN. One study each found no associations between EPA biomarker levels and ACS, AFib, SCD, MI, hemorrhagic stroke, total stroke, cardiac death, CHD death, or total stroke death.

Table EP.3. Evidence profile for the effect and association of EPA, specifically, with CVD outcomes*

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Major adverse cardiovascular events (MACE)	Insufficient	RCT: 1 Obs intake: 1 Obs biomarker: 3	Low	RCT: NA Obs intake: NA Obs biomarker: Inconsistent All: Inconsistent	RCT: Precise Obs intake: Imprecise Obs biomarker: Imprecise	Sparse RCT	RCT: Lower risk Obs intake: Lower risk Obs biomarker: Unclear
CVD death (including stroke)	Insufficient	RCT: 0 Obs intake: 2 Obs biomarker: 1	Low	RCT: NA Obs intake: Inconsistent Obs biomarker: NA All: NA	RCT: NA Obs intake: Precise Obs biomarker: Precise	No RCT	RCT: NA Obs intake: Unclear Obs biomarker: Lower risk
Cardiac death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Unclear	Sparse	RCT: NA Obs intake: NA Obs biomarker: No association
Coronary heart disease death	Insufficient	RCT: 1 Obs intake: 2 Obs biomarker: 1	Low	RCT: NA Obs intake: Consistent Obs biomarker: All: Consistent	RCT: Imprecise Obs intake: Imprecise Obs biomarker: Imprecise	None	RCT: No effect Obs intake: No association Obs biomarker: No association
Myocardial infarction death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Heart failure death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Stroke death	Insufficient	RCT: 1 Obs intake: 1 Obs biomarker: 1	Low	RCT: NA Obs intake: Obs biomarker: All: Consistent	RCT: Imprecise Obs intake: Imprecise Obs biomarker: Imprecise	Sparse	RCT: No effect Obs intake: No association Obs biomarker: No association
Ischemic stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 0	Moderate	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: Precise Obs biomarker: NA	Sparse	RCT: NA Obs intake: Lower risk Obs biomarker: NA
Hemorrhagic stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 0	Moderate	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: NA	Sparse	RCT: NA Obs intake: No association Obs biomarker: NA

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Death, all-cause	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 3	Low	RCT: NA Obs intake: NA Obs biomarker: Inconsistent All: NA	RCT: NA Obs intake: NA Obs biomarker: Precise	No RCT	RCT: NA Obs intake: NA Obs biomarker: Unclear
Coronary heart disease	Low	RCT: 0 Obs intake: 2 Obs biomarker: 3	Low	RCT: NA Obs intake: Yes Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	No RCT	RCT: NA Obs intake: No association Obs biomarker: Unclear
Myocardial infarction	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Precise	Sparse	RCT: NA Obs intake: NA Obs biomarker: No association
Acute coronary syndrome	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: No association Obs biomarker: No association
Angina pectoris	Insufficient	RCT: 1 Obs intake: 0 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: Lower risk Obs intake: NA Obs biomarker: NA
Atrial fibrillation	Low	RCT: 0 Obs intake: 0 Obs biomarker: 3	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Imprecise	No RCT	RCT: NA Obs intake: NA Obs biomarker: No association
Congestive heart failure	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 3	Low	RCT: NA Obs intake: NA Obs biomarker: Consistent All: NA	RCT: NA Obs intake: NA Obs biomarker: Precise	No RCT	RCT: NA Obs intake: NA Obs biomarker: Unclear
Stroke, total	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: NA Obs biomarker: No association
Stroke, ischemic	Insufficient	RCT: 0 Obs intake: 1 Obs biomarkers: 2	Low	RCT: NA Obs intake: NA Obs biomarkers: Consistent All: Consistent	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: No association Obs biomarkers: No association

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Stroke, hemorrhagic	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: NA	Sparse	RCT: NA Obs intake: NA Obs biomarkers: No association
Sudden cardiac death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarkers: 1	Low	RCT: NA Obs intake: NA Obs biomarkers: NA All: NA	RCT: NA Obs intake: NA Obs biomarkers: NA	No RCT	RCT: NA Obs intake: NA Obs biomarkers: No association
Revascularization	Insufficient	RCT: 1 Obs intake: 0 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarker: NA
Hypertension	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Blood pressure (SBP, DBP, MAP combined)	Insufficient	RCT: 2 Obs intake: 0 Obs biomarker: 0	NA	RCT: Inconsistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: NA Obs intake: NA Obs biomarker: NA
LDL-c	Insufficient	RCT: 2 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarker: NA
HDL-c	Insufficient	RCT: 2 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarker: NA
Triglycerides	Insufficient	RCT: 2 Obs intake: 0 Obs biomarker: 0	Low	RCT: Consistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: Lower risk (lower triglycerides) Obs intake: NA Obs biomarker: NA
HDL-c/Total cholesterol to LDL-c ratios	Insufficient	RCT: 1 Obs intake: 0 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: Lower risk Obs intake: NA Obs biomarker: NA

* No reporting bias was detected for any outcome. All studies that measured n-3 FA intake were assessed to be direct, while all biomarker studies were assessed to be indirect.
Abbreviations: DBP = diastolic blood pressure, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MAP = mean arterial pressure, NA = not applicable, Obs = observational study, RCT = randomized controlled trial, SBP = systolic blood pressure.

DHA

For the most part, there is insufficient evidence regarding the effect of, or association with, DHA (specifically) and CVD clinical and intermediate outcomes (**Table EP.4**). There is moderate strength of evidence of no effect of purified DHA supplementation on BP or LDL-c and low strength of evidence of no association between DHA intake and incident CHD (from observational studies).

Clinical event outcomes, RCTs

No trial that reported clinical event outcomes evaluated DHA alone.

Intermediate outcomes, RCTs

Two trials compared purified DHA (3.6 and 2 g/d) to placebo and found no significant effects on systolic or diastolic BP. One of the trials also found no significant effect on MAP. Three trials of DHA (3.7, 3.6, or 2 g/d) also found no significant effect compared to placebo on LDL-c or HDL-c. Two of the trials (3.7 and 3.6 g/d) reported on Tg concentration changes and both found significant net reductions compared to placebo with DHA supplementation (−27 and −29 mg/dL) . The trial of DHA 3.6 g/d also found a significant reduction in Total:HDL-c ratio (−0.3).

Observational studies, intake

Eight studies evaluated the association between estimated total DHA intake (specifically) and risk of clinical outcomes. No study evaluated any outcome in more than two studies. Two studies found significant associations between higher DHA intake and lower risk of incident HTN in healthy young adults (18-30 years old in one study; 39-54 year old women in a subgroup of one study), but not in an older subgroup in one study (55-89 years old). In the study of young adults, a significant association was found in quartiles with DHA intake >0.06 g/d. One of two studies of healthy adults found an association of lower CVD death with DHA intake >0.15 g/d. Two studies each found no association with CHD death or incident CHD (in populations with a broad range of ages, from 20-69 to 45-84 years old). One study each found significant associations of higher DHA intake with MACE (>0.15 g/d DHA), ischemic stroke death (>0.15 g/d), and all-cause death (>0.02 g/d). In one study each, no associations were found with ACS, ischemic stroke, hemorrhagic stroke death, or total stroke death.

Observational studies, biomarkers

Eleven studies evaluated various DHA biomarkers and their associations with clinical outcomes. A high proportion of association analyses were statistically significant favoring higher DHA biomarker levels. Four studies evaluated MACE (with various definitions); two found significant associations between higher DHA biomarker levels (phospholipid and adipose DHA) and lower risk of MACE in healthy adults. The other two studies found no association, one in hypercholesterolemic adults on statins (plasma DHA) and one in healthy adults (erythrocyte DHA). Two of three studies in healthy adults found significant associations between lower CHD risk and higher plasma or phospholipid DHA; the third study, also in healthy adults found no association with blood DHA. Three studies evaluated CHF. One found associations between higher cholesteryl ester and phospholipid DHA and lower risk of incident CHF in healthy women, but not healthy men (whether the associations were significantly different between

women and men was not reported). One study found that overall, there was no significant association with blood DHA in adults with a history of MI, but that there were significant associations in subgroups of people (where the difference in association between subgroups was at least nearly significant), such that significant association were found in people (after MI) not taking a statin (P interaction with statin use = 0.003), ≥65 years old (P interaction = 0.051), with LDL-c ≥100 mg/dL (P interaction = 0.068), and with HDL-c ≤40 mg/dL (P interaction = 0.096). Three studies also evaluated all-cause death, two of which found significantly lower risk of death with higher plasma DHA (healthy adults) and blood DHA (in people with a history of MI who are not taking statins); another study of healthy adults found no association with plasma DHA.

Two studies found near significant associations between higher cholesteryl ester DHA, phospholipid DHA, and plasma DHA and lower risk of ischemic stroke in healthy adults. One of two studies of healthy adults found an association between higher plasma DHA and lower risk of CVD death (both studies evaluated plasma DHA). One study each found significant associations between DHA biomarkers and AFib, SCD, and CHD death (all plasma DHA in healthy adults). One study found a significant association between adipose DHA and ACS in healthy men, but not healthy women. Another study found a significant association between erythrocyte DHA and incident HTN in healthy women aged 39 to 54 years, but not in older women. One study found no significant associations between plasma DHA and both total stroke and total stroke death in healthy adults. One study, each, found no significant associations with MI, hemorrhagic stroke, or cardiac death.

Table EP.4. Evidence profile for the effect and association of DHA, specifically, with CVD outcomes*

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Major adverse cardiovascular events (MACE)	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 4	Low	RCT: NA Obs intake: NA Obs biomarker: Inconsistent All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	No RCT	RCT: NA Obs intake: Unclear Obs biomarker: Unclear
CVD death (including stroke)	Insufficient	RCT: 0 Obs intake: 2 Obs biomarker: 1	Low	RCT: NA Obs intake: Consistent Obs biomarker: NA All: NA	RCT: NA Obs intake: Precise Obs biomarker: Precise	No RCT	RCT: NA Obs: Unclear Obs biomarker: Lower risk
Cardiac death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Unclear	Sparse	RCT: NA Obs intake: NA Obs biomarker: No association
Coronary heart disease death	Insufficient	RCT: 0 Obs intake: 2 Obs biomarker: 1	Low	RCT: NA Obs intake: Consistent Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Precise	No RCT	RCT: NA Obs intake: No association Obs biomarker: Lower risk
Myocardial infarction death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Heart failure death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: No association Obs biomarker: Unclear
Ischemic stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 0	Moderate	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: Precise Obs biomarker: NA	Sparse	RCT: NA Obs intake: Lower risk Obs biomarker: NA
Hemorrhagic stroke death	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 0	Moderate	RCT: NA Obs intake: NA Obs biomarker: All:	RCT: NA Obs intake: Imprecise Obs biomarker: NA	Sparse	RCT: NA Obs intake: No association Obs biomarker: NA
Death, all-cause	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 3	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Precise	No RCT	RCT: NA Obs intake: NA Obs biomarker: Unclear

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Coronary heart disease	Low	RCT: 0 Obs intake: 2 Obs biomarker: 3	Low	RCT: NA Obs intake: Yes Obs biomarker: Inconsistent All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	No RCT	RCT: NA Obs intake: No association Obs biomarker: Unclear
Myocardial infarction	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: Unclear Obs biomarker: NA	Sparse	RCT: NA Obs intake: No association Obs biomarker: NA
Acute coronary syndrome	Insufficient	RCT: 0 Obs intake: 1 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: No association Obs biomarker: NA
Angina pectoris	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Atrial fibrillation	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 1	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Precise	Sparse	RCT: NA Obs intake: NA Obs biomarker: Lower risk
Congestive heart failure	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 3	NA	RCT: NA Obs intake: NA Obs biomarker: Consistent All: NA	RCT: NA Obs intake: NA Obs biomarker: Imprecise	No RCT	RCT: NA Obs intake: NA Obs biomarker: Unclear
Stroke incidence and death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 1	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Imprecise	Sparse	RCT: NA Obs intake: NA Obs biomarker: No association
Ventricular arrhythmia	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Revascularization	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Hypertension	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Blood pressure (SBP, DBP, MAP combined)	Moderate	RCT: 3 Obs intake: 0 Obs biomarker: 0	NA	RCT: Consistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Few studies	RCT: No effect Obs intake: NA Obs biomarker: NA
LDL-c	Moderate	RCT: 3 Obs intake: 0 Obs biomarker: 0	Low	RCT: Consistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Few studies	RCT: No effect Obs intake: NA Obs biomarker: NA
HDL-c	Insufficient	RCT: 3 Obs intake: 0 Obs biomarker: 0	Low	RCT: Inconsistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Few studies	RCT: Unclear Obs intake: NA Obs biomarker: NA
Triglycerides	Insufficient	RCT: 2 Obs intake: 0 Obs biomarker: 0	Low	RCT: Consistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: Lower risk (lower triglycerides) Obs intake: NA Obs biomarker: NA
HDL-c/Total cholesterol to LDL-c ratios	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA

* No reporting bias was detected for any outcome. All studies that measured n-3 FA intake were assessed to be direct, while all biomarker studies were assessed to be indirect.
Abbreviations: DBP = diastolic blood pressure, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MAP = mean arterial pressure, NA = not applicable, Obs = observational study, RCT = randomized controlled trial, SBP = systolic blood pressure.

DPA

Overall, there is insufficient evidence regarding effect of or association between DPA (specifically) and CVD clinical and intermediate outcomes (**Table EP.5**). There is low strength of evidence of an association between higher DPA biomarker levels and lower risk of AFib.

RCTs

No eligible RCTs compared purified DPA formulations versus placebo.

Observational studies, intake

Two observational studies evaluated estimated total DPA intake (specifically). One study found no significant association between DPA intake and ACS in either healthy men or women. The other found significant associations between higher DPA intake and both incident CHD and MACE in healthy adults, in both instances with a significant association in the quartile with DPA intake >0.04 g/d.

Observational studies, biomarkers

Seven studies evaluated the association of various DPA biomarkers with clinical outcomes, all in healthy adults. No outcome was evaluated by more than three studies. One study in adults age ≥65 years was the only study that evaluated several clinical outcomes. It found significant associations between higher plasma DPA and lower risks of all-cause and CVD death, near-significant associations with incident CHF (P=0.057) and total stroke death (P=0.056), but no significant associations with AFib, SCD, hemorrhagic, ischemic, or total stroke, or CHD death. For two outcomes, one of three studies found significant associations; one study found a significant association between blood DPA and incident CHD, but two found no associations with plasma or phospholipid DPA; one study found a significant association between adipose tissue DPA and MACE, but two found no associations with phospholipid or erythrocyte DPA. One study evaluated ACS and found a significant association in men with adipose tissue DPA, but not in women. One study evaluated incident HTN and found a significant association of erythrocyte DPA in younger women (39-54 years old), but not older women (55-89 years old). One study found no significant association with cardiac death.

Table EP.5. Evidence profile for the effect and association of DPA biomarkers, specifically, with CVD outcomes (observational studies only)*

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
MACE	Insufficient	3	Low	Inconsistent	Imprecise	None	Unclear
CVD death (including stroke)	Insufficient	1	Low		Imprecise	None	Lower risk
Cardiac death	Insufficient	1	Low		Unclear	None	No association
Coronary heart disease death	Insufficient	1	Low		Imprecise	None	No association
Myocardial infarction death	Insufficient	0	NA		NA	None	NA
Heart failure death	Insufficient	0	NA		NA	None	NA
Stroke death	Insufficient	1	Low		Imprecise	None	Lower risk
Ischemic stroke death	Insufficient	0	NA		NA	None	NA
Hemorrhagic stroke death	Insufficient	0	NA		NA	None	NA
Death, all-cause	Insufficient	1	Low		Precise	None	Lower risk
Coronary heart disease	Insufficient	3	Low	Inconsistent	Imprecise	None	Unclear
Myocardial infarction	Insufficient	0	NA		NA	None	NA
Acute coronary syndrome	Insufficient	1	Low	NA	Imprecise	Sparse	No association
Angina pectoris	Insufficient	0	NA	NA	NA	NA	NA
Atrial fibrillation	Low	3	Low	Consistent	Imprecise	None	No association
Congestive heart failure	Insufficient	1	Low	NA	Imprecise	Sparse	Lower risk
Stroke incidence and death	Insufficient	1	Low	NA	Precise	Sparse	No association
Ventricular arrhythmia	Insufficient	0	NA	NA	NA	NA	NA
Revascularization	Insufficient	0	NA	NA	NA	None	NA
Hypertension	Insufficient	0	NA	NA	NA	None	NA
Blood pressure (SBP, DBP, MAP combined)	Insufficient	0	NA	NA	NA	None	NA
LDL-c	Insufficient	0	NA	NA	NA	None	NA
HDL-c	Insufficient	0	NA	NA	NA	None	NA
Triglycerides	Insufficient	0	NA	NA	NA	None	NA
HDL-c/Total cholesterol to LDL-c ratios	Insufficient	0	NA	NA	NA	None	NA

* No reporting bias was detected for any outcome. All studies that measured n-3 FA intake were assessed to be direct, while all biomarker studies were assessed to be indirect.
Abbreviations: DBP = diastolic blood pressure, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MACE = major adverse cardiovascular events, MAP = mean arterial pressure, NA = not applicable, SBP = systolic blood pressure.

Marine oil comparisons

There is insufficient evidence regarding comparisons of specific marine oils.

Clinical event outcomes, RCTs

No trial that reported clinical event outcomes compared marine oils.

Intermediate outcomes, RCTs

Two trials that compared marine oil (EPA 3.8 g/d vs. DHA 3.6 g/d; EPA+DHA 3.4 and 1.7 g/d vs. EPA 1.8 g/d) found no significant differences in effect on BP, LDL-c, HDL-c, Tg, or Total:HDL-c ratio.

ALA

There is moderate strength of evidence of no significant effect of ALA intake on BP, LDL-c, HDL-c, or Tg (**Table EP.6**). There is low strength of evidence of no association between ALA intake or biomarker level and CHD or CHD death, AFib, CHF, total and ischemic stroke, each based on observational studies. There is insufficient evidence regarding other outcomes.

Clinical event outcomes, RCTs

Two RCTs that evaluated ALA supplementation versus placebo reported clinical event outcomes, one in participants with CVD and one in healthy participants. All analyses were nonsignificant, for all-cause death (2 trials) and from one trial each, MACE, CVD death, cardiac death, CHD death, CHF death, total MI, incident angina, total stroke, and SCD. Within-study subgroup analyses revealed no significant differences in effect for various subgroups for MACE (1 trial) or with or without diabetes for CHD death (1 trial).

Intermediate outcomes, RCTs

Five ALA RCTs evaluated BP, with doses ranging from 1.4 to 5.9 g/d for 1 to 3.4 years. All found no significant effect on systolic or diastolic BP, mostly with wide confidence intervals. One of the trials found no significant difference in effect on BP between those with hypertension and the study population as a whole. Another trial found no significant difference in effect between 1.4 and 5.9 g/d ALA. No trial reported on MAP.

Four of the trials reported no significant effects of ALA on LDL-c, HDL-c, Tg, or Total:HDL-c ratio (2 trials). No differences in effect were found in the one trial that compared 1.4 and 5.9 g/d ALA. No trial reported on LDL:HDL-c ratio.

Observational studies, intake

Thirteen observational studies evaluated ALA intake. One of these was a pooling of 11 prior studies (the pooled studies are not included in duplicate for the outcomes evaluated by the pooling study). The large majority of analyses found no significant associations; only two studies found any significant associations between higher ALA intake and clinical outcomes. Two studies both found significant associations between higher ALA intake and reduced all-cause death (>2.2 g/d in healthy adults; also in healthy men but insufficient data were reported regarding a dose threshold). One of two studies found a significant association between higher ALA intake (>0.6 g/d) and SCD in healthy women but not in a subset of women with CVD; the second study found no significant association in healthy adults. One of two studies found a

significant association between higher ALA intake (unclear threshold) and lower risk of CVD death in younger men (35-57 years old), but another study found no association in older men (≥65 years old). Among four analyses, representing 14 total studies, only one study (not the pooled study) found a significant association between higher ALA intake and lower CHD death risk (unclear threshold). For all other analyzed clinical outcomes, no significant associations were found with ALA intake, including incident CHD (6 analyses of 16 studies total), CHF (4 studies), CVD (3 studies), MACE (2 studies), hemorrhagic and ischemic stroke (2 studies each), AFib (1 study), and HTN (1 study).

Observational studies, biomarkers

Eight studies evaluated various ALA biomarkers. Almost all analyses found no significant associations between ALA biomarkers and clinical outcomes. No outcome was evaluated by more than three studies. For CHF, one of three studies found a significant association between higher plasma ALA in healthy men, but two other studies found no significant associations in healthy adults with plasma, cholesteryl ester, or phospholipid ALA. One of two studies found a significant association between higher plasma ALA and lower risk of CVD death, but the other study found no significant association also with plasma ALA in healthy adults. No significant associations were found for ischemic stroke (3 studies), incident CHD, hemorrhagic and total stroke (2 studies each), MACE (2 studies), all-cause death (2 studies), or AFib, SCD, incident HTN, cardiac death, or CHD death (1 study each).

SDA

Overall, there is insufficient evidence regarding effect of or association between SDA (specifically) and CVD clinical and intermediate outcomes (**Table EP.7**).

RCTs

No eligible RCTs compared purified SDA formulations versus placebo.

Observational studies

A single eligible observational study in healthy men evaluated baseline erythrocyte SDA and clinical outcomes. Erythrocyte SDA was not significantly associated with either MACE or cardiac death.

Marine oil versus ALA

There is insufficient evidence of direct comparisons between marine oil and ALA intake on CVD outcomes. Across studies, the comparison between marine oil and ALA is unclear, largely because of insufficient evidence regarding ALA; however, where there is high strength of evidence of significant effects of marine oil on improving Tg and HDL-c, there is moderate strength of evidence of no effect of ALA intake on these intermediate outcomes.

Clinical event outcomes, RCTs

No trial that reported clinical event outcomes directly compared marine oils and ALA.

Intermediate outcomes, RCTs

One trial that compared two doses of EPA+DHA (1.7 and 0.8 g/d) with ALA 4.5 g/d found no differences systolic or diastolic BP at 4 months. Across trials, regardless of n-3 FA type, there was no evidence of an effect of BP; no difference in effect was apparent between marine oil and ALA trials.

Two trials that compared EPA+DHA (0.8 and 1.7 g/d in one trial, 0.4 g/d in the other) to ALA (4.5 [rapeseed oil margarine] and 2 g/d ["plant oil" margarine], respectively) for 6 months and 3.4 years found no differences between n-3 FA types for LDL-c, HDL-c, or Tg. Neither trial reported on lipid ratios. No evident differences were found across trials between marine oils and ALA for their nonsignificant effects on LDL-c and HDL-c. In contrast with the two trials that directly compared EPA+DHA and ALA, 32 marine oil (versus placebo) trials fairly consistently found significant effect on Tg reduction in contrast with the four ALA (versus placebo) trials, which mostly had imprecise estimates of effects on Tg.

Table EP.6. Evidence profile for the effect and association of ALA with CVD outcomes*

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Major adverse cardiovascular events (MACE)	Insufficient	RCT: 1 Obs intake: 2 Obs biomarker: 0	Low	RCT: NA Obs intake: Consistent Obs biomarker: All: NA	RCT: Precise Obs intake: Imprecise Obs biomarker: NA	Sparse	RCT: NA Obs intake: 0 Obs biomarker: NA
CVD death (including stroke)	Insufficient	RCT: 1 Obs intake: 2 Obs biomarker: 2	Low	RCT: NA Obs intake: Inconsistent Obs biomarker: Consistent All: NA	RCT: Imprecise Obs intake: Imprecise Obs biomarker: Imprecise	Sparse RCT	RCT: No effect Obs intake: Unclear Obs biomarker: No association
Cardiac death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 1	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: NA Obs intake: NA Obs biomarker: Unclear	Sparse	RCT: NA Obs intake: NA Obs biomarker: No association
Coronary heart disease death	Low	RCT: 1 Obs intake: 4 Obs biomarker: 1	Low	RCT: NA Obs intake: Consistent Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: Imprecise Obs biomarker: Imprecise	Sparse RCT	RCT: No effect Obs intake: No association Obs biomarker: No association
Myocardial infarction death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Heart failure death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Stroke death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Ischemic stroke death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Hemorrhagic stroke death	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Death, all-cause	Insufficient	RCT: 1 Obs intake: 0 Obs biomarker: 2	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: Precise	Sparse	RCT: No effect Obs intake: NA Obs biomarker: No association

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Coronary heart disease	Low	RCT: 0 Obs intake: 6 Obs biomarker: 0	Low	RCT: NA Obs intake: Yes Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: NA	No RCT	RCT: NA Obs intake: No association Obs biomarker: NA
Myocardial infarction	Insufficient	RCT: 1 Obs intake: 0 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: Unclear Obs intake: NA Obs biomarker: NA
Acute coronary syndrome	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All:	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Angina pectoris	Insufficient	RCT: 1 Obs intake: 0 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarker: NA
Atrial fibrillation	Low	RCT: 0 Obs intake: 3 Obs biomarker: 3	Low	RCT: NA Obs intake: Consistent Obs biomarker: NA All: NA	RCT: NA Obs intake: Imprecise Obs biomarker: Imprecise	No RCT	RCT: NA Obs intake: No association Obs biomarker: No association
Congestive heart failure	Low	RCT: 1 Obs intake: 4 Obs biomarker: 3	Low	RCT: NA Obs intake: Consistent Obs biomarker: Consistent All: Consistent	RCT: Imprecise Obs intake: Precise Obs biomarker: Precise	Sparse RCT	RCT: No effect Obs: No association Obs biomarker: Unclear
Stroke incidence and death	Insufficient	RCT: 1 Obs intake: 3 Obs biomarker: 2	Low	RCT: NA Obs intake: Consistent Obs biomarker: Consistent All: Consistent	RCT: Imprecise Obs intake: Imprecise Obs biomarker: Imprecise	Sparse RCT	RCT: No effect Obs intake: Unclear Obs biomarker: No association
Ventricular arrhythmia	Insufficient	RCT: 1 Obs intake: 0 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Imprecise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarker: NA
Revascularization	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
Hypertension	Insufficient	RCT: 0 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
Blood pressure (SBP, DBP, MAP combined)	Moderate	RCT: 4 Obs intake: 0 Obs biomarker: 0	Low	RCT: Consistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Few studies	RCT: No effect Obs intake: NA Obs biomarker: NA
LDL-c	Moderate	RCT: 4 Obs intake: 0 Obs biomarker: 0	Low	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarker: NA
HDL-c	Moderate	RCT: 4 Obs intake: 0 Obs biomarker: 0	Low	RCT: Consistent Obs intake: NA Obs biomarker: NA All: NA	RCT: Precise Obs intake: NA Obs biomarker: NA	Few studies	RCT: No effect Obs intake: NA Obs biomarker: NA
Triglycerides	Moderate	RCT: 3 Obs intake: 0 Obs biomarker: 0	NA	RCT: NA Obs intake: NA Obs biomarker: NA All: NA	NA	No data	RCT: NA Obs intake: NA Obs biomarker: NA
HDL-c/Total cholesterol to LDL-c ratios	Insufficient	RCT: 2 Obs intake: 0 Obs biomarker: 0	Low	RCT: Consistent Obs intake: NA Obs biomarker: NA All:	RCT: Precise Obs intake: NA Obs biomarker: NA	Sparse	RCT: No effect Obs intake: NA Obs biomarker: NA

* No reporting bias was detected for any outcome. All studies that measured n-3 FA intake were assessed to be direct, while all biomarker studies were assessed to be indirect.
Abbreviations: DBP = diastolic blood pressure, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MAP = mean arterial pressure, NA = not applicable, Obs = observational study, RCT = randomized controlled trial, SBP = systolic blood pressure.

Table EP.7. Evidence profile for the effect and association of SDA biomarkers, specifically, with CVD outcomes (observational studies only)*

Outcome	SoE Grade	Design No. Studies	Study Limitations	Consistency	Precision	Other Issues	Finding
MACE	Insufficient	1	Low	NA	Obs biomarker: Unclear	Sparse	No association
CVD death (including stroke)	Insufficient	0	NA	NA	NA	No data	NA
Cardiac death	Insufficient	1	Low	NA	Obs biomarker: Unclear	Sparse	No association
Coronary heart disease death	Insufficient	0	NA	NA	NA	No data	NA
Myocardial infarction death	Insufficient	0	NA	NA	NA	No data	NA
Heart failure death	Insufficient	0	NA	NA	NA	No data	NA
Stroke death	Insufficient	0	NA	NA	NA	No data	NA
Ischemic stroke death	Insufficient	0	NA	NA	NA	No data	NA
Hemorrhagic stroke death	Insufficient	0	NA	NA	NA	No data	NA
Death, all-cause	Insufficient	0	NA	NA	NA	No data	NA
Coronary heart disease	Insufficient	0	NA	NA	NA	No data	NA
Myocardial infarction	Insufficient	0	NA	NA	NA	No data	NA
Acute coronary syndrome	Insufficient	0	NA	NA	NA	No data	NA
Angina pectoris	Insufficient	0	NA	NA	NA	No data	NA
Atrial fibrillation	Insufficient	0	NA	NA	NA	No data	NA
Congestive heart failure	Insufficient	0	NA	NA	NA	No data	NA
Stroke incidence and death	Insufficient	0	NA	NA	NA	No data	NA
Ventricular arrhythmia	Insufficient	0	NA	NA	NA	No data	NA
Revascularization	Insufficient	0	NA	NA	NA	No data	NA
Hypertension	Insufficient	0	NA	NA	NA	No data	NA
Blood pressure (SBP, DBP, MAP combined)	Insufficient	0	NA	NA	NA	No data	NA
LDL-c	Insufficient	0	NA	NA	NA	No data	NA
HDL-c	Insufficient	0	NA	NA	NA	No data	NA
Triglycerides	Insufficient	0	NA	NA	NA	No data	NA
HDL-c/Total cholesterol to LDL-c ratios	Insufficient	0	NA	NA	NA	No data	NA

* No reporting bias was detected for any outcome. All studies that measured n-3 FA intake were assessed to be direct, while all biomarker studies were assessed to be indirect.
Abbreviations: DBP = diastolic blood pressure, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MACE = major adverse cardiovascular events, MAP = mean arterial pressure, NA = not applicable, SBP = systolic blood pressure.

Chapter 4. Discussion

Overall summary of key findings

In this systematic review we identified 55 eligible RCTs (in 85 publications) and 33 eligible prospective longitudinal and nested case-control studies (in 59 publications) for inclusion based on prespecified eligibility criteria. Most of the RCTs evaluated the effects of marine oil supplements (EPA+DHA) compared with placebo on clinical CVD outcomes in populations at risk for CVD or with CVD, while most of the observational studies examined the associations between intake of various individual n-3 FA and in combination with each other in relationship to long-term CVD events in generally healthy populations. The RCTs of intermediate CVD outcomes (BP and lipids) were conducted in all three populations of interest (generally healthy, at risk for CVD—primarily due to dyslipidemia, or with CVD). However, none of the observational studies evaluated BP or lipids.

The main findings of the studies, regarding effect or association of increased n-3 FA intake or biomarker level and outcomes are summarized in the following tables. **Table Disc.1** includes analyses of n-3 FA and outcome pairs for which there is evidence supporting an effect or association of increased n-3 FA intake and lower risk of a CVD outcome or an improved cardiovascular risk factor.

Table Disc.1. Main findings of high, moderate, or low strength of evidence of significant effects or associations between n-3 FA and outcomes

There is **high** strength of evidence for the following effects or associations of *increased* n-3 FA intake or biomarker levels and *lower* cardiovascular risks or events:

- Marine oil supplementation (or increased intake) and an increase in HDL-c
  - RCTs (of mostly supplements)
  - Summary net change in HDL-c: 1.2 mg/dL (95% CI 0.6, 1.8)
- Marine oil supplementation (or increased intake) and a decrease in triglycerides (Tg)
  - RCTs (of mostly supplements)
  - Summary net change in Tg: -23 mg/dL (95% CI -29, -18)
- Marine oil supplementation (or increased intake) and a decrease in total or LDL-c to HDL-c ratio
  - RCTs (of mostly supplements)
  - Summary net change in LDL:HDL-c ratio: −0.3 (95% CI −0.4, −0.1)

There is **moderate** strength of evidence for the following effects or associations of *increased* n-3 FA intake or biomarker levels and *lower* cardiovascular risks or events:

- Marine oil supplementation (or increased intake) and a lower risk of major adverse cardiovascular events (MACE)
  - RCTs (of mostly supplements); however, observational studies found no association
  - Summary effect size (RCTs): 0.95 (95% CI 0.90, 1.00)
- Marine oil supplementation (or increased intake) and a possibly lower risk of cardiovascular disease (CVD) death
  - RCTs (of mostly supplements); however, observational studies found no association
  - Summary effect size (RCTs): 0.91 (95% CI 0.81, 1.01)

There is **low** strength of evidence for the following effects or associations of *increased* n-3 FA intake or biomarker levels and *lower* cardiovascular risks or events:

- Marine oil increased intake and a lower risk of coronary heart disease (CHD)
  - Observational studies (of total dietary intake), supported by a single study of n-3 FA biomarkers
- Marine oil increased intake (up to about 0.2 g/d) and a lower risk of congestive heart failure (CHF); no association between intake and CHF risk for intakes >0.2 g/d
  - Observational studies (of total dietary intake); however RCTs of supplements found no effect and biomarker associations studies found no association
  - Summary HR (per g/d): 0.45 (95% CI 0.28, 0.72) (observational studies) for intake between about 0 and 0.2 g/d

Table Disc.2 includes analyses of n-3 FA and outcome pairs for which there is evidence supporting no effect or association of n-3 FA intake (or biomarker level) and outcomes. Analyses of n-3 FA and outcome pairs not included in the boxes provided insufficient evidence.

Table Disc.2. Main findings of high, moderate, or low strength of evidence of no significant effects or associations between n-3 FA and outcomes

There is **high** strength of evidence of *no effect or association* of n-3 FA intake or biomarker level and the following outcomes:

- Marine oil (long-chain n-3 FA, mostly EPA and DHA) intake and all-cause death
  - RCTs (of mostly supplements) supported by observational studies (of total dietary intake)
- Marine oil intake and total stroke (fatal and nonfatal ischemic and hemorrhagic stroke)
  - RCTs (of mostly supplements) supported by observational studies (of total dietary intake)
- Marine oil intake and sudden cardiac death (SCD)
  - RCTs (of mostly supplements) supported by an observational study (of total dietary intake)
- Marine oil intake and coronary revascularization
  - RCTs (of mostly supplements) supported by an observational study (of total dietary intake)
- Marine oil intake and systolic or diastolic blood pressure
  - RCTs (of mostly supplements)
- Marine oil intake and LDL-c
  - RCTs (of mostly supplements)

There is **moderate** strength of evidence of *no effect or association* of n-3 FA intake or biomarker level and the following outcomes:

- Marine oil intake and myocardial infarction
  - RCTs (of mostly supplements) supported by an association study (of total dietary intake)
- Marine oil intake and atrial fibrillation
  - RCTs (of mostly supplements); observational studies of intake were inconsistent
- Purified DHA supplementation and systolic or diastolic blood pressure
  - RCTs
- Purified DHA supplementation and LDL-c
  - RCTs
- ALA intake and systolic or diastolic blood pressure
  - RCTs (of mostly supplements)
- ALA intake and lipoproteins (LDL-c, HDL-c) or Tg
  - RCTs (of mostly supplements)

There is **low** strength of evidence of *no effect or association* of n-3 FA intake or biomarker level and the following outcomes:

- Total n-3 FA intake and stroke death
  - Observational studies (of total dietary intake)
- Total n-3 FA intake and myocardial infarction death
  - Observational studies (of total dietary intake)
- Marine oil intake and CHD death
  - RCTs (of mostly supplements); observational studies of intake were inconsistent
- Marine oil intake and ischemic or hemorrhagic strokes
  - Observational studies (of total dietary intake)
- EPA intake and CHD
  - Observational studies (of total dietary intake)
- EPA biomarkers and atrial fibrillation
  - Observational studies
- DHA intake and CHD
  - Observational studies (of total dietary intake)
- DPA biomarkers and atrial fibrillation
  - Observational studies
- ALA intake and CHD or, separately, CHD death
  - Observational studies (of total dietary intake); CHD death finding supported by one RCT (of supplementation)
- ALA intake and atrial fibrillation

    - Observational studies (of total dietary intake)
- ALA intake and CHF
    - Observational studies (of total dietary intake), supported by one RCT (of supplementation)

Studies within each category of analysis (by study design and by n-3 FA) were diverse, due to differences in outcomes evaluated, definitions of specific outcomes, as well as the n-3 FA intervention doses or compositions (for RCTs) or the dietary/biomarker n-3 FA exposure assessments and quantifications (for observational studies). Overall we found a lack of conclusive or consistent findings for CVD events within RCTs, mostly due to sparse data and underpowered trials as indicated by wide confidence intervals. The majority of the individual RCTs did not find statistically significant effects of marine oil supplements (EPA+DHA, various doses) on CVD outcomes. Pooled meta-analyses suggest that people with CVD or at risk for CVD who received marine oil supplements may have a small risk reduction in MACE (pooled HR 0.95, 95% CI 0.90, 1.00) and possibly in CVD death (pooled HR 0.91, 0.81, 1.01) compared with those who received placebo. The effects of marine oil supplements were often larger in earlier RCTs than in more recent RCTs. These data may be confounded by shifts over time in concomitant therapy to reduce CVD risk (e.g., statins). Observational studies were mixed regarding the associations between n-3 FA intake or biomarkers and risk of MACE (where each study used its own combination of specific CVD outcomes). The strength of associations between higher levels of n-3 FA and lower risk of CVD outcomes, when found, were often larger than those in RCTs. While all observational studies adjusted associations for potentially confounding variables, the specific variables included in models varied greatly across observational studies. Furthermore, all observational studies compared higher intake levels of n-3 FA with lowest intake level, which included people who may have other nutrition deficiencies that may affect chronic disease risks but often cannot be "controlled for" in the analyses (resulting in residual, uncontrolled confounding).

The overall findings for the effects of marine oil supplements on intermediate CVD outcomes remain largely unchanged since the original report. In this update, there were no significant effects found in 22 RCTs that compared marine oils (0.3-6 g/d) on SBP or DBP compared with placebo. Thirty-three RCTs evaluated LDL-c and HDL-c. Meta-analysis of the effect of marine oils on LDL-c found no significant effect. In contrast, marine oils increased HDL-c by a small, statistically significant amount (summary net change = 1.2 mg/dL; 95% CI 0.6, 1.8). The clinical significance of this small increase in HDL-c on CVD outcomes is unclear. For both lipids, no differences in effect across studies were found by marine oil dose, followup duration or population. The strongest effect of marine oils (0.3-6 g/d) was found among the 34 RCTs of Tg. Meta-analysis found a summary net change of −23 mg/dL (95% CI −29, −18), with no significant difference in effect based on population or followup time across studies. However, across trials, the effect was dose-dependent and also dependent on the studies' mean baseline Tg values. By metaregression, each increase of EPA+DHA dose by 1 g/d was also associated with a greater net change Tg of −6.8 mg/dL (95% CI −11.4, −2.2) and each increase in mean baseline Tg level by 1 mg/dL was associated with a greater net change Tg of −0.12 mg/dL (95% CI −0.22, −0.03). However, the few trials that directly compared marine oil doses did not consistently find consistently find a dose effect; although, marine oil doses ≥3 g/d all resulted in larger reductions in Tg compared to lower doses, in contrast to doses <3 g/d which had smaller reductions in Tg compared to even lower doses. There were no observational studies evaluating these intermediate CVD outcomes.

In the original report, there was only one RCT of ALA (linseed oil) versus control oil (sunflower seed oil),[137] conducted in the 1960s, that evaluated clinical event outcomes. In this update we identified only one additional RCT of ALA (plant source not reported) versus placebo (oleic acid) in participants with a history of MI that reported clinical outcomes.[114] Given the sparseness of trials of the effect on clinical CVD outcomes of increased ALA intake and the differences between the two trials, no conclusion can be drawn regarding effect of ALA on CVD outcomes. For intermediate outcomes, five ALA RCTs (with doses ranging from 1.4 to 5.9 g/d) evaluated BP outcomes, and four of the five RCTs also evaluated LDL-c, HDL-c, Tg, or Total:HDL-c ratio (2 trials) outcomes. All found no significant differences in these outcomes between ALA and placebo. Thirteen observational studies evaluated ALA intake. The large majority of analyses found no significant associations; only two studies found any significant associations between higher ALA intake and clinical outcomes (reduced all-cause death, SCD, and CHD death risks).

The potential threshold-effects of n-3 FA on CVD events could not be determined from the RCTs because there were limited number of RCTs for many outcomes and most RCTs did not find significant effects. Using data from observational studies, the linear dose-response and potential threshold effects of n-3 FA on several CVD events were tested by meta-analytical techniques. There was a near significant association between EPA and DHA intake and CHD across a median dose range of 0.04 to 3.47 g/d (effect size per g/d = 0.90 [95% CI 0.80, 1.01]), and a just-significant association between EPA and DHA intake and *higher* risk of ischemic stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 1.03 [95% CI 1.00, 1.07]), but no dose-response relationships found between EPA and DHA intake and hemorrhagic stroke. The interpretations of the threshold-effects were limited because differences in associations at lower doses (statistically significant associations between higher intake and lower risk) and associations at higher doses (no significant associations between intake and outcome) were generally similar regardless of the cut point chosen between lower and higher dose analyses.

No differences in effects or associations were found between different populations (healthy or general population, at increased risk for CVD—largely due to dyslipidemia, or with CVD). However, this conclusion is weak given that few studies compared populations, few RCTs were conducted in healthy populations and few observational studies were conducted in at risk or CVD populations.

Limitations

Overall, both RCTs and observational studies (i.e., longitudinal observational and nested case-control studies) included in this systematic review generally had few risk of bias concerns. Across RCTs, the most common risk of bias limitation was a lack of intention-to-treat analyses (25% of the included RCTs). Of included RCTs, 18 percent could not blind study participants because the intervention was dietary (increased fish intake, not n-3 FA supplements), and 15 percent of RCTs were at risk of attrition bias primarily due to overall dropout rates greater than 20 percent. Most studies reported similar dropout rates between groups. Although more than 90 percent of the included RCTs reported similar baseline demographic characteristics between groups, about 40 percent did not report baseline n-3 FA intake or status. This is a critical point because baseline n-3 FA status likely affects response to changes in n-3 FA intake (diet or supplements). Across observational studies, the most common risk of bias limitation was reporting inadequacy related to the ranges and distribution of n-3 FA exposures (45% did not fully report such data). Of included observational studies, 12 percent did not report the dietary

assessment instrument, and most of the n-3 FA dietary intake assessment included only dietary sources (not n-3 FA supplements). Of those studies that reported biomarker data, this is not an issue of concern. However, a variety of different n-3 FA biomarkers were investigated across studies, making comparisons and meta-analysis difficult.

For clinical CVD outcomes, all but one of the RCTs was conducted in either high risk individuals or people with existing CVD. In contrast, most observational studies examining the associations between dietary n-3 FA intake or biomarkers of n-3 FA intake and clinical outcomes were conducted in generally healthy populations. The definitions of most clinical outcomes were heterogeneous across studies regardless of the study designs. For most clinical outcomes, there were few or no RCTs. Few trials compared n-3 FA dose, formulation, or source. No trial compared different n-3 to n-6 FA ratios of supplements or intake. None of the observational studies attempted to determine a threshold effect of any associations between n-3 FA and the outcome of interest.

Other study-reporting issues that precluded analyses from being included in meta-analyses were that studies of n-3 FA intake used a variety of methods to measure intake (g/d, percent Kcal, percent fat or fatty acid intake); several studies failed to report median or range data of n-3 FA levels within quantiles, confidence intervals (or equivalent) of association hazard ratios, or conducted only linear analyses across a full range of n-3 FA values. In addition, studies varied in the range of n-3 FA status (e.g., intake level) within each study, often with n-3 FA ranges that did not or hardly overlapped. All of the observational studies measured dietary n-3 FA intake or biomarkers of n-3 FA intake at a single time point, baseline, and related these data to the long-term (mostly >10 years) clinical outcomes (CVD events). These analyses rely on the assumption that baseline intake reflects long-term intake, both prior to the beginning of the study and during the course of the observational period. In adults, the relative stability of dietary patterns may minimize the bias due to changing dietary patterns. However, study participants may have changed their dietary or supplement intake of n-3 FA due to concerns about CVD, due to advancing age or new CVD risk factors (e.g., new diagnoses of HTN, diabetes, or dyslipidemia). These potential dietary changes are unlikely to have occurred at random and may, therefore, introduce bias due to the differential misclassifications of exposure status.

There are numerous differences between RCTs and observational studies, making the comparisons across the two study designs difficult to make. Of note, the doses of marine oil supplements (EPA+DHA) in RCTs were often much higher than the highest intake reported for observational studies. Furthermore, not all observational studies explicitly included n-3 FA supplements in their assessment of intake and very few of the RCTs attempted to account for background fish or n-3 FA intake as an effect modifier.

Due to significant clinical heterogeneity across studies, the interpretation of overall meta-analysis results is limited. Dose-response meta-analysis of observational studies should be interpreted with caution as many factors many invalidate the results such as heterogeneity in the covariate adjustments across studies and errors or biases in dietary assessments.

While this report represents a complete systematic review, it does not encompass all trials or longitudinal observational studies that report on CVD and intermediate outcomes. Particularly, if one includes small studies (trials with <30 participants per study group or observational studies with <100 participants, several hundred more studies could potentially have met eligibility criteria. Due to time and resource limitations, we restricted the review to the approximately 100 studies that are most likely to have adequately addressed the primary research questions of interest.

Future research recommendations

Future RCTs should clearly characterize the preparations of n-3 FA, both as individual FA composition and sources of n-3 FA and control oils. It is preferable that standardized n-3 FA oils are analyzed to allow clearer interpretation of what the interventions are and the association between specific n-3 FA and CVD effects. Researchers are encouraged to use standard, common CVD outcomes to allow comparison across studies. The potential biomarkers of status and intake should be evaluated at the study entry and post-intervention in all study participants. Subject recruitment criteria should consider using narrow ranges of n-3 FA status and demographic characteristics so that the effect of the n-3 FA intervention can be evaluated in the absence of known confounders. The effects (or lack thereof) of marine oils (EPA+DHA) on BP, lipoproteins, and Tg are well established so additional RCTs on these intermediate outcomes alone are unlikely to add any new knowledge, and therefore are not needed.

Observational studies would benefit from more consistent and precise assessment methods for establishing n-3 FA status and the use of more consistent approaches to assess outcomes. There is an ongoing need to improve self-reported dietary assessment methods and food databases for all nutrients including n-3 FA. As national dietary patterns shift and new processed foods are introduced into the marketplace, food frequency questionnaires need to be updated to ensure accurate estimation of n-3 FA (and other nutrient) intake. Similar to trial registries, a data repository for raw observational study data would greatly improve the transparency of data analyses (potentially reduce both reporting and publication biases) and the appropriateness and methodology of meta-analytical techniques for pooling observational studies. An individual participant-level meta-analysis of observational studies of marine oils could address limitations of the study-level meta-analyses that are currently feasible.

Conclusions

Results from the RCTs of clinical event outcomes are applicable only to at risk of CVD and CVD populations. Results from the RCTs of intermediate outcomes are applicable to all populations. In contrast, results from observational studies (which did not evaluate intermediate outcomes) are applicable only to generally healthy populations. We graded the strength of the body of evidence for each intervention/exposure and comparison of intervention, and for each outcome by assessing the number of studies, their study designs, the study limitations (i.e., risk of bias and overall methodological quality), the directness of the evidence to the Key Questions, the consistency of study results, the precision of any estimates of effect, the likelihood of reporting bias, and the overall findings across studies. We concluded that there is insufficient evidence regarding the effect of or association between total n-3 FA (ALA + marine oils [EPA+DHA+DPA) and clinical or intermediate outcomes. There is low strength of evidence of no association between total n-3 FA intake and stroke death, and total (fatal and nonfatal) MI (each association based on longitudinal observational studies). For marine oil (EPA+DHA±DPA), there is insufficient evidence for most outcomes of interest but there is low to high strength of evidence of a beneficial effect of increased marine oil intake for selected CVD and intermediate outcomes. Specifically, there is high strength of evidence of that marine oils clinically and statistically significantly lower Tg—possibly with greater effects with higher doses and in people with higher baseline Tg—and statistically, but arguably not clinically, significantly raises HDL-c. There is also high strength of evidence that marine oil significantly lowers Total:HDL-c ratio. There is moderate strength of evidence that marine oil supplementation lowers risk of MACE events and total CVD death. There is a high strength of

evidence of no effect of marine oil on risk of total stroke (ischemic and hemorrhagic, fatal and nonfatal), but low strength of evidence of no associations of marine oil intake and ischemic or hemorrhagic stroke. There is low strength of evidence for associations between higher EPA+DHA intake and decreased risk of CHD and CHF, based on observational studies. However, there is moderate to high strength of evidence of no effect of (or association between) marine oil and all-cause death, MI, AFib, CHF, sudden cardiac death, revascularization, BP, LDL-c, or LDL:HDL-c ratio. There is also low strength of evidence of no effect of marine oil intake and CHD death.

For individual n-3 FA, there is insufficient evidence regarding the effect of, or association with, EPA, DHA, DPA, SDA, or ALA (specifically) and most CVD clinical outcomes. For EPA, there is low strength of evidence of no association between EPA intake and CHD and between EPA biomarkers and AFib. For DHA, there is moderate strength of evidence of no effect of purified DHA supplementation on BP or LDL-c and low strength of evidence of no association between DHA intake and incident CHD (from observational studies). For DPA (no RCT was identified), there is low strength of evidence of an association between higher DPA biomarker levels and lower risk of AFib. For ALA, there is moderate strength of evidence of no significant effect of ALA intake on BP, LDL-c, HDL-c, or Tg. There is low strength of evidence of no association between ALA intake or biomarker level and CHD or CHD death, AFib, CHF, total and ischemic stroke, based on observational studies.

There is insufficient evidence of direct comparisons between marine oil and ALA intake on CVD outcomes. Across studies, the comparison between marine oil and ALA is unclear, largely because of insufficient evidence regarding ALA; however, where there is high strength of evidence of significant effects of marine oil on improving Tg and HDL-c, there is moderate strength of evidence of no effect of ALA intake on these intermediate outcomes. No RCTs examined the additive effects of n-3 FA versus the effects of individual n-3 FA.

In the scientific community, there is a perception of "conflicting evidence" for the role of n-3 FA in prevention or treatment of CVD between RCT and observational study data.[190, 191] This perception may in part stem from inconsistent scientific conclusions among several of the expert panels or may relate to whether the potential beneficial effects of n-3 FA were from fish (or other marine foods) intake or from dietary supplements.[4-7] Our qualitative comparisons between RCTs and observational studies (i.e., longitudinal observational and nested case-control studies) included in this systematic review showed that the evidence base from the two study designs relating n-3 FA to CVD outcomes often are not comparable as they address different research questions. It is important to note that observational studies of fish consumption without quantifications of n-3 FA were not included in this systematic review. Our findings highlight the importance of including both observational studies and RCTs to assess the strength of body of evidence because the two study designs each have their own strengths and weakness and often provide complementary pieces of information for causal inferences. Nutrition observational studies typically measure and compare people with different dietary behaviors (thus different levels of nutrient exposure) in relationship to the disease risks, while nutrition RCTs are typically designed to compare a specific (usually narrowly defined) nutrition intervention to a control) in a relatively homogenous and well-defined study population. By design, nutrition observational studies and RCTs address different research questions. The observed relationships between higher or lower levels of intake and disease risks are important to describe potential behavioral target for interventions for prevention or treatment of a disease but will never be sufficient to pin point the specific mechanism or doses for the interventions. Therefore it is unlikely that a RCT

can be designed to “verify” or “validate” nutrition observational results. On the other hand, RCTs are the most valid design for comparative effectiveness research questions. Long-term nutrition RCTs, however, often suffer compliance or contamination issues that can void the advantages of initial randomization. No single study can provide a “definitive answer” due to the unique challenges in nutrition RCTs and observational studies. It is necessary to carefully review the totality of evidence while considering the strengths and limitations of the individual studies.

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